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PTO/SB/21 (05-03)
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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Issued Patent	**6,887,861**
Issued Date	**May 3, 2005**
Application Number	**09/707,730**
Filing Date	**November 6, 2000**
First Named Inventor	**HILL, GORDON CRAIG**
Group Art Unit	**1647**
Examiner Name	**LANDSMAN, ROBERT S.**
Total Number of Pages in This Submission: **56**	Attorney Docket Number: **ASIL-002CIP**

ENCLOSURES (check all that apply)

- ☐ Fee Transmittal Form
- ☒ **Credit Card Payment Form**
- ☐ Amendment / Reply
  - ☐ After Final
  - ☐ Affidavits/declaration(s)
- ☐ Extension of Time Request
- ☐ Express Abandonment Request
- ☐ Information Disclosure Statement
- ☐ Certified Copy of Priority Documents
- ☐ Response to Missing Parts/ Incomplete Application
  - ☐ Response to Missing Parts under 37 CFR 1.52 or 1.53
- ☐ Assignment Papers *(for an Application)*
- ☐ Drawing(s)
- ☐ Licensing-related Papers
- ☐ Petition
- ☐ Petition to Convert to a Provisional Application
- ☐ Power of Attorney, Revocation Change of Correspondence Address
- ☐ Terminal Disclaimer
- ☐ Request for Refund
- ☐ CD, Number of CD(s
- ☐ After Allowance Communication to Group
- ☐ Appeal Communication to Board of Appeals and Interferences
- ☐ Appeal Communication to Group *(Appeal Notice, Brief, Reply Brief)*
- ☒ **Petition for Certificate of Correction (4 pgs.)**
- ☒ **Certificate of Correction (3 pgs.)**
- ☒ Other Enclosure(s) *(please identify below):*

Copy of Issued Patent with changes (12 pgs.)

Copy of Application with changes (19 pgs.)

Copy of Amendment filed on 9.28.04 (16 pgs.)

Postcard

Remarks

Certificate of Correction SEP 28 2005

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Signing Attorney/Agent (Reg. No.)	**EDWARD J. BABA, 52,581** **BOZICEVIC, FIELD & FRANCIS, LLP**
Signature	
Date	**September 20, 2005**

EXPRESS MAIL LABEL NO. EV 687 633 420 US

This collection of information is required by 37 CFR 1.5. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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SEP 28 2005

EXPRESS MAIL LABEL NO. **EV 687 633 420 US**

PETITION FOR CERTIFICATE OF CORRECTION

Attorney Docket	ASIL-002CIP
First Named Inventor	HILL, GORDON
Patent Number	6,887,861
Issue Date	May 3, 2005
Application Number	09/707,730
Filing Date	November 6, 2000
Title: *"COMPOUNDS FOR INTRACELLULAR DELIVERY OF THERAPEUTIC MOIETIES TO NERVE CELLS"*	

Address to:
Mail Stop DAC
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Applicants petition under 37 C.F.R. § 1.322 for a Certificate of Correction to correct errors in the claims and specification for the above-identified patent due to Patent and Trademark Office error and due to typographical errors in the application as filed.

Transmitted herewith for filing is a Certificate of Correction for the above-identified patent. Please make the following corrections:

In column 2, line 52, the word "hot" should be replaced with the word -- not --.
In column 2, line 58, the word "t hat" should be replaced with the word -- that --.
In column 3, line 13, the word "etenolol" should be replaced with the word -- atenolol --.
In column 3, line 48, the word "quartinery" should be replaced with the word -- quaternary --.
In column 3, line 49, the word "quartinery" should be replaced with the word -- quaternary --.
In column 3, line 51, the word "quartinery" should be replaced with the word -- quaternary --.
In column 4, line 67, the word "quartinery" should be replaced with the word -- quaternary --.
In column 5, line 1, the word "quartinery" should be replaced with the word -- quaternary --.
In column 5, line 3, the word "quartinery" should be replaced with the word -- quaternary --.
In column 5, line 10, the word "Moieities" should be replaced with the word -- moieties --.
In column 6, line 7, the word "etenolol" should be replaced with the word -- atenolol --.
In column 6, line 19, the word "thiolether" should be replaced with the word -- thioether --.
In column 6, line 56, the word "acidlabile" should be replaced with the word -- acid labile --.
In column 7, line 47, the word "here" should be replaced with the word -- where --.
In column 8, line 33, the word "gancyclovir" should be replaced with the word -- ganciclovir --.

SEP 28 2005

In column 9, line 62, the letter "A" should be replaced with the word -- Angstrom --.

In column 11, line 9, the word "etenolol" should be replaced with the word -- atenolol --.

In column 11, line 12, the word "gancyclovir" should be replaced with the word -- ganciclovir --.

In column 11, line 49, the word "quartinery" should be replaced with the word -- quaternary --.

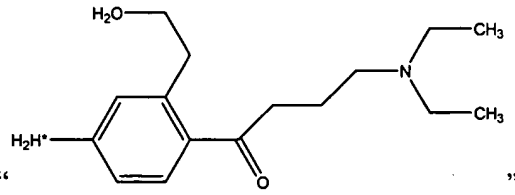
In column 11, line 50, the word "quartinery" should be replaced with the word -- quaternary --.

In column 11, line 52, the word "quartinery" should be replaced with the word -- quaternary --.

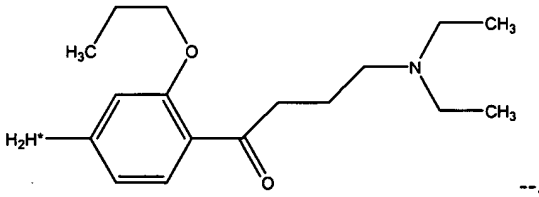
In column 12, line 35, the word "alkylse" should be replaced with the word -- alkyls --.

In column 15, line 44, the word "neurotrohin" should be replaced with the word -- neurotrophin --.

In column 17, lines 26-35, the structure for propoxycaine "



" should be replaced with structure --



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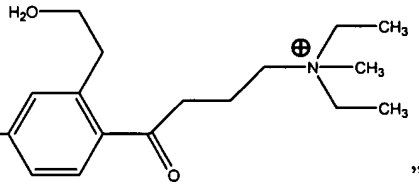
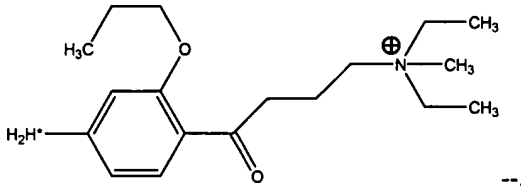
In column 17, lines 28-44, the structure for quaternary propoxycaine derivative



" should be replaced with structure --



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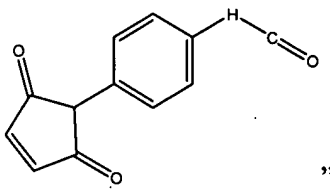
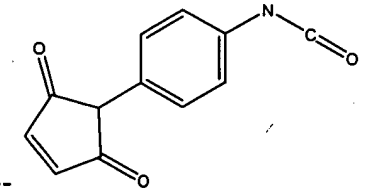
In column 21, Table 4, the word "PMM" should be replaced with the word -- PMPI --.

In column 21, Table 4, the structure "  " should be replaced with the structure --  --.

In column 26, Table 4, the word "is" should be replaced with the word -- in --.

In column 26, Table 4, the word "NGP" should be replaced with the word -- NGF --.

In column 27, Table 4, the words "Synth Tic" should be replaced with the word -- Synthetic --.

In column 28, Table 4, the word "MGF" should be replaced with the word -- NGF --.

In column 28, Table 4, the words "19.2 dinelep" should be replaced with the words -- 19.2 nmoles --.

In column 28, Table 4, the word "Trsur's" should be replaced with the word -- Traut's --.

In column 29, Table 4, the word "arcyclovir" should be replaced with the word -- acyclovir --.

In column 30, Table 4, the word "Centerlfoge" should be replaced with the word -- Centrifuge --.

In column 37, line 11, the word "herein" should be replaced with the word -- wherein --.

In column 37, line 64, the word "herein" should be replaced with the word -- wherein --.

In column 38, line 1, the word "herein" should be replaced with the word -- wherein --.

Enclosed is a copy of the specification filed on November 6, 2000, showing the correct language and a copy of the Amendment filed on September 28, 2004 showing the correct language of the claims. Also enclosed, is a copy of the pages of the issued patent showing the incorrect language.

Commissioner is hereby authorized to charge any fees under 37 C.F.R. § 1.20, which may be required by this paper, or to credit any overpayment, to Deposit Account No. 50-0815 order number ASIL-002CIP.

Respectfully submitted,
BOZICEVIC, FIELD & FRANCIS LLP

By: _____
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Registration No. 52,581

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PTO/SB/44 (02-01)
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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

(Also form PTO-1050)

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO : 6,887,861
DATED : May 3, 2005
INVENTOR(S) : HILL, GORDON CRAIG, et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In column 2, line 52, the word "hot" should be replaced with the word -- not --.
In column 2, line 58, the word "t hat" should be replaced with the word --that--.
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In column 12, line 35, the word "alkylse" should be replaced with the word --alkyls--.
In column 15, line 44, the word "neurotrohin" should be replaced with the word --neurotrophin--.

MAILING ADDRESS OF SENDER:

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PATENT NO. 6,887,861

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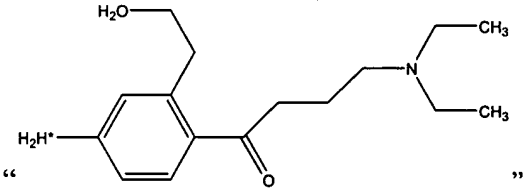

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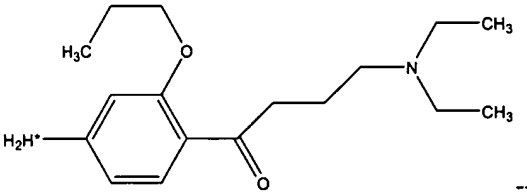

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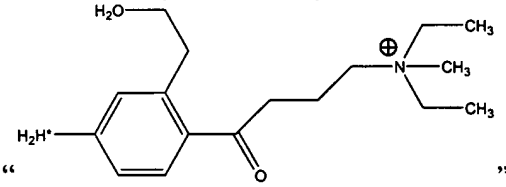

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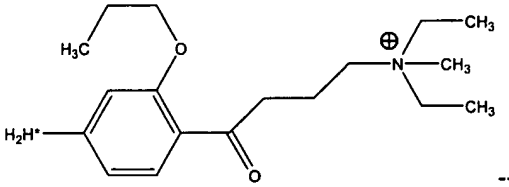

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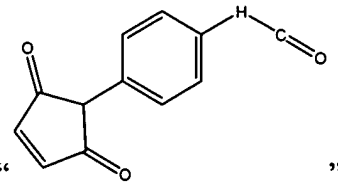
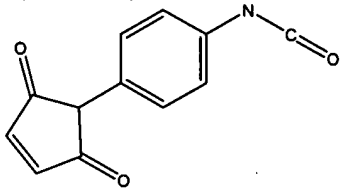
In column 21, Table 4, the structure "  " should be replaced with the structure --  --.

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East Palo Alto, California 94303

PATENT NO. 6,887,861

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COMPOUNDS FOR INTRACELLULAR DELIVERY OF THERAPEUTIC MOIETIES TO NERVE CELLS

RELATIONSHIP TO COPENDING APPLICATIONS

This application is a continuation-in-part of copending U.S. application Ser. No. 09/217,037 entitled *COMPOUNDS FOR INTRACELLULAR DELIVERY OF THERAPEUTIC MOIETIES TO NERVE CELLS*, filed Dec. 21, 1998, which is incorporated herein by reference. U.S. application Ser. No. 09/217,037 issued as U.S. Pat. No. 6,652,864.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to compounds which can be used to selectively deliver moieties to nerve cells. More specifically, the invention relates to compounds which include a therapeutic moiety and facilitate absorption of the therapeutic moiety by nerve cells.

2. Description of Related Art

Our understanding of the structure and function of the nervous system has been greatly advanced owing to enormous progresses made in field of neuroscience. Cellular and molecular mechanisms of neuron growth and development and diseases associated with the central and peripheral nervous systems are studied extensively by using rapidly growing techniques in molecular and cell biology. However, a need still exists for efficacious treatments of many neurological disorders including Alzheimer's disease, Parkinson's disease, Huntington's disease, schizophrenia, severe pain, multiple sclerosis, bipolar disease, and diseases of the nervous system due to infection by viruses and other microorganisms (herpes simplex, HIV, cytomegalovirus, parasites, fungi, prion, etc.).

Many neuropharmaceutical agents have been developed to treat diseases of the nervous system, but their usefulness has been hampered by severe side effects partially due to nonspecific interactions between these agents and cells or tissues other than the targeted cells. For example, steroid hormone cortisone and its derivatives are widely used to treat inflammation in the body including the nerve system to reduce symptoms such as swelling, tenderness and pain. However, the steroid dosage has to be kept at the lowest effective level because of its severe side effects. Steroid hormone binds to its cognate nuclear hormone receptor and induces a cascade of cellular effects, including programmed cell death of the neurons in the brain (Kawata M., et al., J. Steroid Biochem. Mol. Biol. 65: 273–280 (1998)). Since steroid hormone receptors, such as glucocorticord receptor for cortisone, distribute in a wide variety of tissues and cells, nonspecific interactions of the hormone with its cognate receptor in different sites is unavoidable if the drug is circulated systemically.

A need continues to exist for an effective system for delivering therapeutic agents selectively to nerve cells and nerve tissues. Various techniques have been developed to deliver drugs, but with only limited success. For example, liposomes have been used as carrier molecules to deliver a broad spectrum of agents including small molecules, DNAs, RNAs, and proteins. Liposome mediated delivery of pharmaceutical agents has major drawbacks because of its lack of target specificity. Attempts have been made to overcome this problem by covalently attaching whole site-specific

antibody or Fab fragments to liposomes containing a pharmaceutical agent (Martin et al., Biochem. 20, 4229–4238, (1981)). However, an intrinsic problem of particular importance in any liposome carrier system is that in most cases the targeted liposome does not selectively reach its target site in vivo. Whether or not liposomes are coated with antibody molecules, liposomes are readily phagocytosed by macrophages and removed from circulation before reaching their target sites.

SUMMARY OF THE INVENTION

Compounds of the present invention include compounds having the general formula:

B-L-M

where:

B is a binding agent capable of selectively binding to a nerve cell surface receptor and mediating absorption of the compound by the nerve cell;

M is a moiety which performs a useful non-cytotoxic function when absorbed by a nerve cell; and

L is a linker coupling B to M.

In one embodiment, the compounds have the general formula:

B-L-TM

where:

B is a binding agent capable of selectively binding to a nerve cell surface receptor and mediating absorption of the compound by the nerve cell;

TM is a therapeutic moiety which has a non-cytotoxic therapeutic effect when absorbed by a nerve cell; and

L is a linker coupling B to TM.

In another embodiment, the compounds have the general formula:

B-L-IM

where:

B is a binding agent capable of selectively binding to a nerve cell surface receptor and mediating absorption of the compound by the nerve cell;

IM is a non-cytotoxic imaging moiety which can be used to image a nerve cell or an intracellular component of the nerve cell; and

L is a linker coupling B to IM.

In regard to each of the above embodiments, particular classes of binding agents B which may be used include, but are not limited to, nucleic acid sequences, peptides, peptidomimetics, antibodies and antibody fragments. Examples of nucleic acids that can serve as the binding agent B include, but are not limited to, DNA and RNA ligands that function as antagonists of nerve growth factors or inhibit binding of other growth factors to nerve cell surface receptors. Examples of peptides that can serve as the binding agent B include, but are not limited to, members of the nerve growth factors (neurotrophin) family such as NGF, BDNF, NT-3, NT-4, NT-6; derivatives, analogs, and fragments of nerve growth factors such as recombinant molecules of NGF and BDNF; and synthetic peptides that bind to nerve cell surface receptors and have agonist or antagonist activities of nerve growth factors.

Antibodies, derivatives of antibodies and antibody fragments can also serve as the binding agent B. Examples of

this type of binding agent B include, but are not limited to, anti-human trk monoclonal antibody 5C3 and anti-human p75 monoclonal antibody MC192.

The therapeutic moiety TM is selected to perform a non-cytotoxic therapeutic function within nerve cells. Examples of non-cytotoxic functions which the therapeutic moiety TM may perform include, but are not limited to, the functions performed by adrenergic agents, analgesics, anti-trauma agents, anti-viral agents, gene therapy agents, and hormones (growth factors, interferons, etc.). Examples of classes of therapeutic moieties include, but are not limited to, adrenergic agents (e.g., epinephrine, norepinephrine, dopamine, etenolol), analgesics (e.g., opioids, codeine, oxycodone), anti-trauma agents, anti-viral agents (e.g., acyclovir, gancyclovir, AZT, ddI, ddC, etc.), gene therapy agents (e.g., DNAs or RNAs which introduce a gene or replace a mutated gene), steroids (e.g., cortisone, progesterone, estrogen), and hormones (e.g., growth factors, interferons).

In one particular embodiment, the therapeutic moiety TM is a charged moiety. Cells have difficulty transporting charged molecules across cell membranes. According to this embodiment, the binding agent B serves to facilitate transport of a charged therapeutic moiety TM into a cell. Within the cell, the compound (i.e. the conjugate formed between B and TM) is metabolized to form a metabolite product that comprises the charged therapeutic moiety TM. The metabolite product is less prone to being transported across the cell membrane out of the cell relative to the conjugate because of the metabolism of the conjugate resulting in the separation of the therapeutic moeity TM from the binding agent B. The metabolite product is also less prone to being transported across the cell membrane out of the cell relative to a non-charged version of the therapeutic moiety due to the charge which the therapeutic moiety carries.

According to this embodiment, compounds are provided which comprise a charged derivative of a therapeutic agent having a therapeutic activity, the charged derivative being conjugated to a protein having a biological activity of being transported across a cell membrane into a cell, the cell metabolizing at least a portion of the protein to form a charged metabolite product that possesses the therapeutic activity of the therapeutic agent, the charged metabolite product being less prone to being transported across the cell membrane out of the cell relative to the conjugate and less prone to being transported across the cell membrane out of the cell relative to the therapeutic agent.

In one particular embodiment, the charged therapeutic moiety TM is a quartinery alkyl amine derivative of a therapeutic moiety A particular example of a quartinery alkyl amine derivative of a therapeutic moiety TM is a quartinery alkyl amine of propoxycaine, shown in Table 3.

The imaging moiety IM is a non-cytotoxic agent which can be used to locate and optionally visualize a nerve cell or an internal component of the nerve cell which has absorbed the imaging moiety. Fluorescent dyes may be used as an imaging moiety IM. Radioactive agents which are non-cytotoxic may also be an imaging moiety IM.

In general, the linker may be any moiety which can be used to link the binding agent B to the moiety M. In one particular embodiment, the linker is a cleavable linker. The use of a cleavable linker enables the moiety M linked to the binding agent B to be released from the compound once absorbed by the nerve cell. The cleavable linker may be cleaved by a chemical agent, enzymatically, due to a pH change, or by being exposed to energy. Examples of forms of energy which may be used include light, microwave, ultrasound, and radiofrequency.

The present invention also relates to a method for selectively delivering a moiety into nerve cells comprising the steps of:

delivering to a patient a compound having the general formula:

B-L-M

where:

B is a binding agent capable of selectively binding to a nerve cell surface receptor and mediating absorption of the compound by the nerve cell;

M is a moiety which performs a useful non-cytotoxic function when absorbed by a nerve cell; and

L is a linker coupling B to M.

having the compound selectively bind to a nerve cell surface receptor via the binding agent B; and

having the compound be absorbed by the nerve cell mediated by the binding of the binding agent B to the nerve cell surface receptor.

In one embodiment, moiety M is a therapeutic moiety TM as described herein and in another embodiment is an imaging moiety IM.

The above method can be used to deliver therapeutic moieties for treating a variety of neurological disorders when the therapeutic moiety TM is a moiety useful for treating such neurological disorders.

The above method can be used to deliver therapeutic moieties for treating pain when a therapeutic moiety TM for treating pain, such as an analgesic, is included as the therapeutic moiety TM in the compound.

The above method can also be used to deliver steroid hormones for treating nerve damage when a therapeutic moiety TM for treating nerve damage, such as a steroid hormone, is included as the therapeutic moiety TM in the compound.

The above method can also be used to stimulate nerve growth when a therapeutic moiety TM for inducing the production of a nerve growth factor is included as the therapeutic moiety TM in the compound.

The above method can also be used to treat infected nerve cells infected with viruses or immunize nerve cells from viruses when the therapeutic moiety TM in the compound is an antiviral agent.

The above method can also be used to perform gene therapy when the therapeutic moiety TM is a gene therapy agent.

The present invention also relates to a method for improving intracellular administration of a therapeutic agent. The method comprises contacting cells with a compound comprising a charged derivative of a therapeutic agent having a therapeutic activity, the charged derivative being conjugated to a protein having a biological activity of being transported across a cell membrane into a cell; and having the cell transport the compound into the cell where the cell metabolizes at least a portion of the protein to form a charged metabolite product that possesses the therapeutic activity of the therapeutic agent, the charged metabolite product being less prone to being transported across the cell membrane out of the cell relative to the conjugate and less prone to being transported across the cell membrane out of the cell relative to the therapeutic agent.

In one embodiment, this method is used in conjunction with the conjugates of the present invention and hence is used in conjunction with the methods of the present invention for selectively delivering a moiety into nerve cells.

In one particular embodiment, the charged therapeutic moiety TM is a quartinery alkyl amine derivative of a

therapeutic moiety A particular example of a quartinery alkyl amine derivative of a therapeutic moiety TM is a quartinery alkyl amine of propoxycaine, shown in Table 3.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to compounds which include a binding agent which binds to a nerve cell surface receptor and facilitates absorption of the compound by the nerve cell; and a moiety. Different Moieties may be included in the compounds of the present invention including therapeutic moieties that are non-cytotoxic to the nerve cells and imaging moieties which can be used to image nerve cells which absorb these compounds.

In one embodiment, compounds of the present invention have the general formula:

B-L-TM

where:

B is a binding agent capable of selectively binding to a nerve cell surface receptor and mediating absorption of the compound by the nerve cell;

TM is a therapeutic moiety which has a non-cytotoxic therapeutic effect when absorbed by a nerve cell; and

L is a linker coupling B to TM.

According to this embodiment, the binding agent B serves as a homing agent for nerve cells by selectively binding to nerve cell surface receptors. The binding agent B also serves to facilitate absorption of the compound by the nerve cell. The binding agent B can be any molecule which can perform these two functions. Particular classes of binding agents which may be used include, but are not limited to, nucleic acid sequences, peptides, peptidomimetics, antibodies and antibody fragments.

Examples of nucleic acids that can serve as the binding agent B include, but are not limited to, DNA and RNA ligands that function as antagonists of nerve growth factors or inhibit binding of other growth factors to nerve cell surface receptors (Binkley, J., et al., Nucleic Acid Res. 23: 3198–3205 (1995); Jellinek, D., et al., Biochem. 33:10450–10456 (1994)).

Examples of peptides that can serve as the binding agent B include, but are not limited to, members of the nerve growth factors (neurotrophin) family such as NGF, BDNF, NT-3, NT-4, NT-6, etc. (see reviews: Frade, J. M., et al., Bioessays 20: 137–145 (1998); Shieh, P. B., Curr. Biol. 7: R627–R630 (1997); Dechant, G., et al., Curr. Opin. Neurobiol. 7: 413–418 (1997); Chao, M. V. and Hempstead, B. L., Trends Neurobiol. 18: 321–326 (1995)); and derivatives, analogs, and fragments of nerve growth factors such as recombinant molecules of NGF and BDNF (Ibanez et al., EMBO J. 10: 2105–2110; Ibanez et al., EMBO J. 12: 2281–2293), synthetic peptides that bind to nerve cell surface receptors and have agonist or antagonist activities of nerve growth factors (Longo, F. M., et al., Cell Regulation 1: 189–195 (1990); LeSauteur, L. et al., J. Biol. Chem. 270: 6564–6569 (1995); Longo F. M., et al., J. Neurosci. Res. 48: 1–17; Longo, et al., Nature Biotech. 14: 1120–1122 (1997)).

Examples of antibodies, derivatives of antibodies and antibody fragments that can serve as the binding agent B include, but are not limited to, anti-human trkA monoclonal antibody 5C3 (Kramer, K., et al., Eur. J. Cancer 33: 2020–2091 (1997)), anti-human p75 monoclonal antibody MC192 (Maliatchouk, S. and Saragovi, H. U., J. Neurosci. 17: 6031–7).

According to this embodiment, the therapeutic moiety TM is selected to perform a non-cytotoxic therapeutic function within nerve cells. Examples of non-cytotoxic functions which the therapeutic moiety TM may perform include, but are not limited to, the functions performed by analgesics, anti-trauma agents, anti-viral agents, gene therapy agents, and hormones (growth factors, interferons, etc.). Examples of classes of therapeutic moieties include, but are not limited to, adrenergic agents (e.g., epinephrine, norepinephrine, dopamine, etenolol), analgesics (e.g., opioids, codeine, oxycodone), anti-trauma agents, anti-viral agents (e.g., acyclovir, gancyclovir, AZT, ddI, ddC, etc.), gene therapy agents (e.g., DNAs or RNAs which introduce a gene or replace a mutated gene), steroids (e.g., cortisone, progesterone, estrogen), and hormones (e.g., growth factors, interferons).

The linker L serves to link the binding agent B to the therapeutic moiety TM. A wide variety of linkers are known in the art for linking two molecules together, particularly, for linking a moiety to a peptide or nucleic acid, all of which are included within the scope of the present invention.

Examples of classes of linkers that may be used to link the binding agent B to the therapeutic moiety TM include amide, alkylamine, thiolether, alkyl, cycloalkyl, aryl linkages such as those described in Hermanson, G. T., Bioconjugate Techniques (1996), Academic Press, San Diego, Calif.

In certain applications, it is desirable to release the therapeutic moiety TM once the compound has entered the nerve cell, resulting in a release of the therapeutic moiety TM. Accordingly, in one variation, the linker L is a cleavable linker. This enables the therapeutic moiety TM to be released from the compound once absorbed by the nerve cell. This may be desirable when the therapeutic moiety TM has a greater therapeutic effect when separated from the binding agent. The therapeutic moiety TM may have a better ability to be absorbed by an intracellular component of the nerve cell when separated from the binding agent. Accordingly, it may be necessary or desirable to separate the therapeutic moiety TM from the compound so that the therapeutic moiety TM can enter the intracellular compartment.

Cleavage of the linker releasing the therapeutic moiety may be as a result of a change in conditions within the nerve cells as compared to outside the nerve cells, for example, due to a change in pH within the nerve cell. Cleavage of the linker may occur due to the presence of an enzyme within the nerve cell which cleaves the linker once the compound enters the nerve cell. Alternatively, cleavage of the linker may occur in response to energy or a chemical being applied to the nerve cell. Examples of types of energies that may be used to effect cleavage of the linker include, but are not limited to light, ultrasound, microwave and radiofrequency energy.

The linker L used to link the binding agent B to the therapeutic moiety TM may be a photolabile linker. Examples of photolabile linkers include those linkers described in U.S. Pat. Nos. 5,767,288 and 4,469,774. The linker L used to link the binding agent B to the therapeutic moiety TM may also be an acid labile linker. Examples of acid labile linkers include linkers formed by using cis-aconitic acid, cis-carboxylic alkatriene, polymaleic anhydride, and other acidlabile linkers, such as those linkers described in U.S. Pat. Nos. 5,563,250 and 5,505,931.

Further examples of cleavable linkers include, but are not limited to the linkers described in Lin, et al., J. Org. Chem. 56:6850–6856 (1991); Ph.D. Thesis of W.-C. Lin, U. C. Riverside, (1990); Hobart, et al., J. Immunological Methods 153: 93–98 (1992); Jayabaskaran, et al., Preparative Biochemistry 17(2): 121–141 (1987); Mouton, et al., Archives of Biochemistry and Biophysics 218: 101–108 (1982); Funkakoshi, et al., J. of Chromatography 638:21–27 (1993); Gildea, et al., Tetrahedron Letters 31: 7095–7098 (1990); WO 85/04674; and Dynabeads (Dynal, Inc., 5 Delaware Drive, Lake Success, N.Y. 11042).

In another embodiment, compounds of the present invention have the general formula:

B-L-IM

where:

B is a binding agent capable of selectively binding to a nerve cell surface receptor and mediating absorption of the compound by the nerve cell;

IM is a non-cytotoxic imaging moiety which can be used to image the nerve cell or an intracellular component of the nerve cell; and

L is a linker coupling B to IM.

According to this embodiment, the binding agent B and linker L may be varied as described above with regard to compounds having the general formula B-L-TM. Further according to this embodiment, the imaging moiety IM may be a non-cytotoxic moiety which can be used to image nerve cells. Examples of imaging moieties that may be used include fluorescent dyes and radioisotopes which are non-cytotoxic.

The present invention also relates to a method for selectively delivering a non-cytotoxic therapeutic moiety into nerve cells comprising the steps of:

delivering to a patient a therapeutic amount of a compound having the general formula:

B-L-TM

where:

B is a binding agent capable of selectively binding to a nerve cell surface receptor and mediating absorption of the compound by the nerve cell,

TM is a therapeutic moiety which has a non-cytotoxic therapeutic effect when absorbed by a nerve cell, and

L is a linker coupling B to TM;

having the compound selectively bind to a nerve cell surface receptor via the binding agent B; and

having the compound be absorbed by the nerve cell mediated by the binding of the binding agent B to the nerve cell surface receptor.

The method of the present invention offers the advantage of specifically targeting a non-cytotoxic therapeutic moiety to nerve cells where the therapeutic moiety is absorbed by the nerve cells. The method utilizes the fact that internalization of the conjugated agent is mediated by the binding of the binding agent B to nerve cell surface receptors. Once internalized, the therapeutic moiety can accumulate within the nerve cells here it has a therapeutic effect. The ability to selectively deliver the compound to nerve cells reduces the overall amount of therapeutic moiety which needs to be administered. Selective delivery of the therapeutic moiety to the nerve cell reduces the amount of side effects observed due to non-specific administration of the therapeutic moiety. In addition, the therapeutic moiety is less likely to be separated from the binding agent and non-specifically administered as compared to delivery methods involving the use of a binding agent and a therapeutic moiety in combination.

The method of the present invention can be used to deliver therapeutic moieties for treating a variety of neurological disorders including, but not limited to, Alzheimer's disease, Parkinson's disease, multiple sclerosis, neurodegenerative disease, epilepsy, seizure, migraine, trauma and pain. Examples of neuropharmaceuticals that may be used include proteins, antibiotics, adrenergic agents, anticonvulsants, nucleotide analogs, anti-trauma agents, peptides and other classes of agents used to treat or prevent a neurological disorders. For example, analgesics such as opioids, codeine and oxycodone can be conjugated to the binding agent B and specifically delivered to the nerve cells. Since the same level of pain relief can be achieved using a smaller dosage of analgesics, side effects such as respiratory depression or potential drug addiction can be avoided or at least ameliorated. Steroid hormones such as corticosteriods can also be conjugated with nerve cell-specific binding agents and used to treat inflammation of the nerves, which may reduce the side effects associated with high doses of steroids, such as weight gain, redistribution of fat, increase in susceptibility to infection, and avascular necrosis of bone.

The method according to the present invention can also be used to deliver agents that induce the production of nerve growth factor in the target nerve cells, especially under conditions of pathogenic under-expression of NGFs (See Riaz, S. S. and Tomlinson, D. R. Prog. Neurobiol. 49: 125–143 (1996)). NGF induction has been demonstrated in a wide variety of cell types, such as fibroblasts (Furukawa, Y. et al., FEBS Lett. 247: 463–467(1989)), astrocytes (Furukawa, Y. et al., FEBS Lett. 208: 258–262 (1986)), Schwann cells (Ohi, T. et al., Biochem. Int. 20:739–746 (1990)) with a variety of agents including cytokines, steriods, vitamins, hormones, and unidentified components of serum. Specific examples of agents known to induce NGF include 4-methylcatechol, clenbuterol, isoprenaline, L-tryptophan, 1,25-dihydroxyvitamin D3, forskolin, felbutamide A, gangliosides and quinone derivatives (Riaz, S. S. and Tomlinson, D. R. Prog. Neurobiol. 49: 125–143 (1996)).

The method according to the present invention can also be used to deliver antiviral drugs into nerve cells in order to treat diseases caused by viral infection, to eliminate viruses spread to the nerves, and to inhibit infection by such viruses. Examples of viruses that infect the nervous system include but are not limited to rabies viruses, herpes viruses, polioviruses, arboviruses, reoviruses, pseudorabies, corona viruses, and Borna disease viruses. For example, antiviral drugs such as acyclovir, gancyclovir, and Cifodovir can be conjugated to the binding agent and used to inhibit active or latent herpes simplex viruses in the peripheral and central nervous system. Specific delivery of the conjugate containing these antiviral drugs to the nervous system can reduce the side effects associated with high doses or long-term administration of these drugs, such as headaches, rash and paresthesia.

The method according to the present invention can also be used to deliver marker compounds to image intracellular components of the nerve cells. Such marker compounds include but are not limited to fluorescent dyes, radioactive complexes, and other luminophores.

The method according to the present invention can also be used to perform gene therapy wherein nucleic acids (DNA or RNA) are delivered to the nerve cells. These nucleic acids may serve to replace genes which are either defective, absent or otherwise not properly expressed by the patient's nerve cell genome.

The above and other features and advantages of the present invention will become more apparent in the following description of the preferred embodiments in greater detail.

1. Binding Agent (B)

According to the present invention, a compound with a binding agent B is used to selectively deliver the conjugated therapeutic moieties TM to nerve cells. At the nerve cell, the binding agent B interacts with a receptor on the nerve cell and is absorbed by the nerve cell mediated by this interaction. Any molecules possessing these two physical properties are intended to fall within the scope of a binding agent B as it is used in the present invention. In particular, peptides or proteins with these features can serve as a binding agent B, examples including but not limited to nerve growth factors (neurotrophins), antibodies against nerve cell-specific surface proteins, mutants and synthetic peptides derived from these peptides or proteins.

2. Therapeutic Moiety (TM)

An aspect of the present invention relates to the delivery of compounds into nerve cells which are non-cytotoxic to the nerve cells and perform a therapeutic function. Examples of therapeutic functions include, but are not limited to, treatment of neurological disorders, gene therapy, intracellular target imaging, cell sorting, or separation schemes. Examples of classes of therapeutic moieties include, but are not limited to adrenergic agents such as epinephrine, norepinephrine, dopamine, tetenolol; analgesics such as opioids, codeine, oxycodone; anti-trauma agents; anti-viral agents such as acyclovir, gancyclovir, AZT, ddI, ddC; gene therapy agents such as; steroids such as cortisone, progesterone, estrogen; and hormones such as growth factors and interferons. Such compounds may optionally also include an imaging moiety, such as fluorescent moieties, for imaging intracellular components of the nerve cells.

A further aspect of the present invention relates to compositions and methods for improving the delivery of a therapeutic agent having a therapeutic activity intracellularly. This is accomplished by using therapeutic moieties which are charged. Cells have difficulty transporting charged molecules across cell membranes. According to this embodiment, the binding agent B serves to facilitate transport of a charged therapeutic moiety TM into a cell. Within the cell, the compound (i.e. the conjugate formed between B and TM) is metabolized to form a metabolite product that comprises the charged therapeutic moiety TM. The metabolite product is less prone to being transported across the cell membrane out of the cell relative to the conjugate because of the metabolism of the conjugate resulting in the separation of the therapeutic moeity TM from the binding agent B. The metabolite product is also less prone to being transported across the cell membrane out of the cell relative to a non-charged version of the therapeutic moiety due to the charge which the therapeutic moiety carries.

According to this embodiment, compounds are provided which comprise a charged derivative of a therapeutic agent having a therapeutic activity, the charged derivative being conjugated to a protein having a biological activity of being transported across a cell membrane into a cell, the cell metabolizing at least a portion of the compound to form a charged metabolite product that possesses the therapeutic activity of the therapeutic agent, the charged metabolite product being less prone to being transported across the cell membrane out of the cell relative to the compound and less prone to being transported across the cell membrane out of the cell relative to the therapeutic agent.

In one particular embodiment, the charged therapeutic moiety TM is a quartinery alkyl amine derivative of a therapeutic moiety. A particular example of a quartinery alkyl amine derivative of a therapeutic moiety TM is a quartinery alkyl amine of propoxycaine, shown in Table 3.

Also according to this embodiment, methods are provided which comprise administering a therapeutic agent to a patient in a form where the therapeutic agent comprises a charge and is conjugated to a protein having the biological activity of being transported across a cell membrane into a cell. Once within the cell, the cell metabolizes at least a portion of the compound to form a metabolite product that possesses the therapeutic activity of the therapeutic agent. The metabolite product is less prone to being transported across the cell membrane out of the cell relative to the compound because of the metabolism of the compound resulting separation of the therapeutic moiety from the protein, and is less prone to being transported across the cell membrane out of the cell relative to an uncharged version of the therapeutic agent.

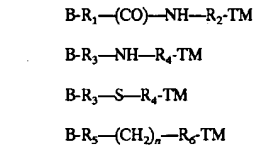
This method may be used in conjunction with the conjugates of the present invention for selectively delivering a moiety to nerve cells. However, it is noted that charged therapeutic moieties can be used with so binding agents that target cells other than nerve cells.

3. Linker (L)

According to the present invention, a binding agent B is linked to a therapeutic moiety TM by a linker L. In general, any method of linking a binding agent to a therapeutic moiety may be used and is intended to fall within the scope of the present invention.

Many different types of linkers have been developed for cross linking proteins and conjugating proteins or peptides with other agents. These linkers include zero-length cross linkers, homobifunctional cross-linkers, heterobifunctional cross-linkers and trifunctional cross-linkers. These linkers may have different susceptibility to cleavage under certain conditions. Depending on a particular application according to the present invention, an appropriate linker may be chosen. When an intracellular release of the agent from its conjugate is desired, a cleavable linker is chosen which is susceptible to cleavage by external stmuli such as light and heat, by intracellular enzymes, or by a particular microenvironment inside the cell.

In one embodiment, the linker L has one of the following general structures:



Wherein R_1, R_2, R_3, R_4, R_5, and R_6 are independently selected from the group consisting of alkylse aryls, heteroaryls, cycloalkyls, cycloalkenes and heterocycloalkenes.

4. Cleavable Linkers

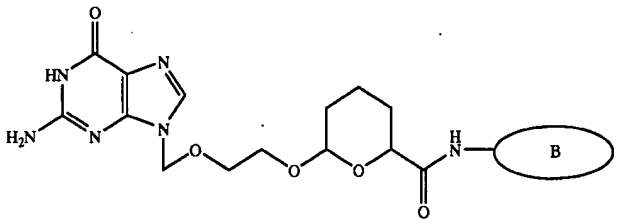
One particular embodiment of the present invention relates to compounds which include a cleavable linker L. In some instances, the therapeutic moiety TM is more efficacious or potent when free from a carrier molecule such as a binding agent. In such instances, it is desirable to utilize a cleavable linker which allows the therapeutic moiety TM to be released from the compound once inside the cell.

Many cleavable linker groups have been developed which are susceptible to cleavage and by a wide variety of mechanisms. For example, linkers have been developed which may be cleaved by reduction of a disulfide bond, by irradiation of a photolabile bond, by hydrolysis of derivatized amino acid side chain, by serum complement-mediated hydrolysis, and by acid-catalyzed hydrolysis.

Examples of photolabile linkers that may be used include those linkers described in U.S. Pat. Nos. 5,767,288 and 4,469,774.

Acid-labile linkers are preferred in the practice of the present invention by taking advantage of a cell's receptor-mediated endocytosis pathways. Receptors that are internalized by receptor-mediated endocytosis pass through acidified compartments known as endosomes or receptosomes. Since the interior of the endosomal compartment is kept acidic (pH~6.0) by ATP-driven H^+ pumps in the endosomal membrane that pump H^+ into the lumen from the cytosol, a change in pH within the nerve cell can be used to cause the acid-labile linker to be cleaved and release the therapeutic moiety. Examples of acid labile linkers which may be used include the cis-aconitic acid, cis-carboxylic alkatriene, poly-

TABLE 2-continued



Acyclovir

wherein

B is selected from the group consisting of nerve growth factors NGF, BDNF, NT-3, NT-4, NT-6, anti-neurotrophin receptor antibodies MAb 5C3 and Mab MC192.

6. Examples of Compounds for Treating Pain

Table 3 provides several therapeutic moieties which may be used in the compounds and methods of the present invention for treating pain. It is noted that any of the various binding moieties and linkers described herein may be employed with these therapeutic agents. Indicated in the table below as * are preferred moieties for attaching linkers to the therapeutic moieties.

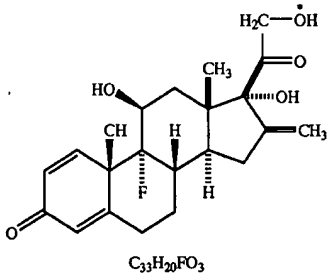
7. Examples of Linkers

Table 4 provides a series of linkers for linking different therapeutic moieties and binding moieties together. As illustrated, linkers are provided for attaching moieties which have thiol (—SH), hydroxyl (—OH), and amino (—NH2) groups to the linkers. In these examples, neurotrophin is shown as the binding agent. However, it is noted that neurotrophin and these examples are intended to be exemplary only. Other linkers may also be used and are intended as part of the present invention.

TABLE 3

Pain - Steroidal anti-inflammatory agents

Betamethazone

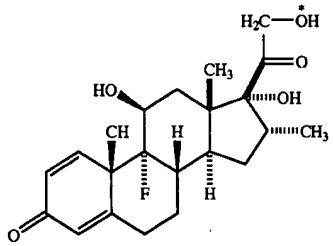


$C_{33}H_{20}FO_3$

Mol. Wt.: 392.47

TABLE 3-continued

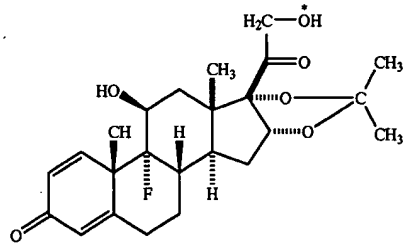
Dexamethazone



$C_{23}H_{29}FO_3$

Mol. Wt.: 392.47

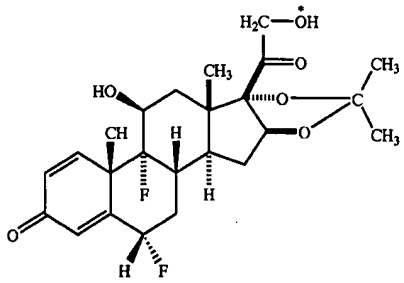
Triamcinolone acetonide



$C_{34}H_{31}FO_6$

Mol. Wt.: 434.51

Fluocinolone acetonide



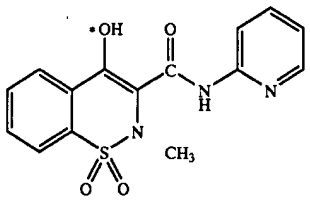
$C_{14}H_{20}F_2O_4$

Mol. Wt.: 452.50

TABLE 3-continued

Pain - Non-steroidal anti-inflamatory agent

Piroxicam

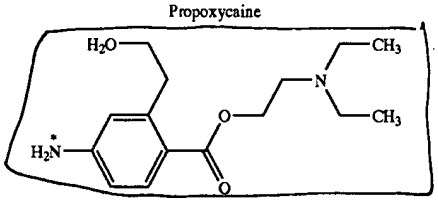


$C_{13}H_{13}N_3O_4S$

Mol. Wt.: 331.35

Pain - Local anesthetic agents

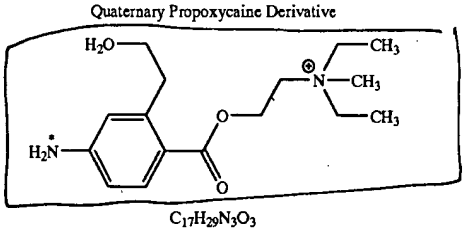
Propoxycaine



$C_{18}H_{28}N_2O_3$

Mol. Wt.: 278.39

Quaternary Propoxycaine Derivative

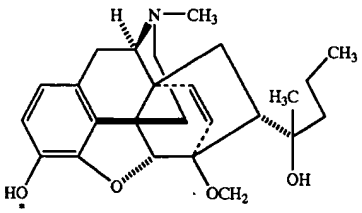


$C_{17}H_{29}N_3O_3$

Mol. Wt.: 293.42

Pain - Narcotic Agonists

Etorphine

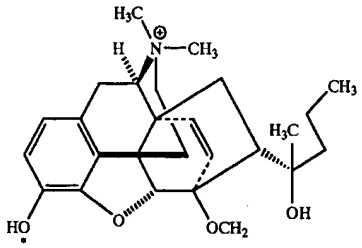


$C_{29}H_{33}NO_4$

Mol. Wt.: 411.54

TABLE 3-continued

Quaternary Etorphine Derivative

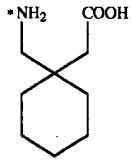


$C_{14}H_{36}NO_4$

Mol. Wt.: 426.57

Pain - Channel blockers

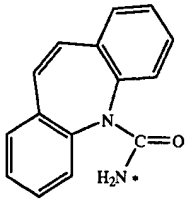
Gabapentin



$C_9H_{17}NO_2$

Mol. Wt.: 171.24

Carbamazepine

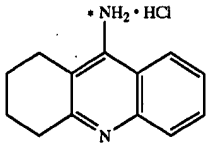


$C_{15}H_{12}N_3O$

Mol. Wt.: 236.27

Anti-neurodegenerative

Tacrine HCl



$C_{13}H_{14}N_2HCl$

Mol. Wt.: 234.73

TABLE 4

Hydroxyl group conjugations
e.g., Steroids, Piroxicam, Acyclovir, Etorphin s

PMM

$C_{11}H_6N_3O_3$ Mol. Wt.: 214.18 Spacer Arm = 8.7 Å

Amino group conjugations
e.g., Propoxycaines, Gabapentin, Carbemazepine, Tacrine

LC-SPDP

$C_{18}H_{11}N_5O_5S_3$ Mol. Wt.: 415.52 Spacer Arm = 15.7 Å

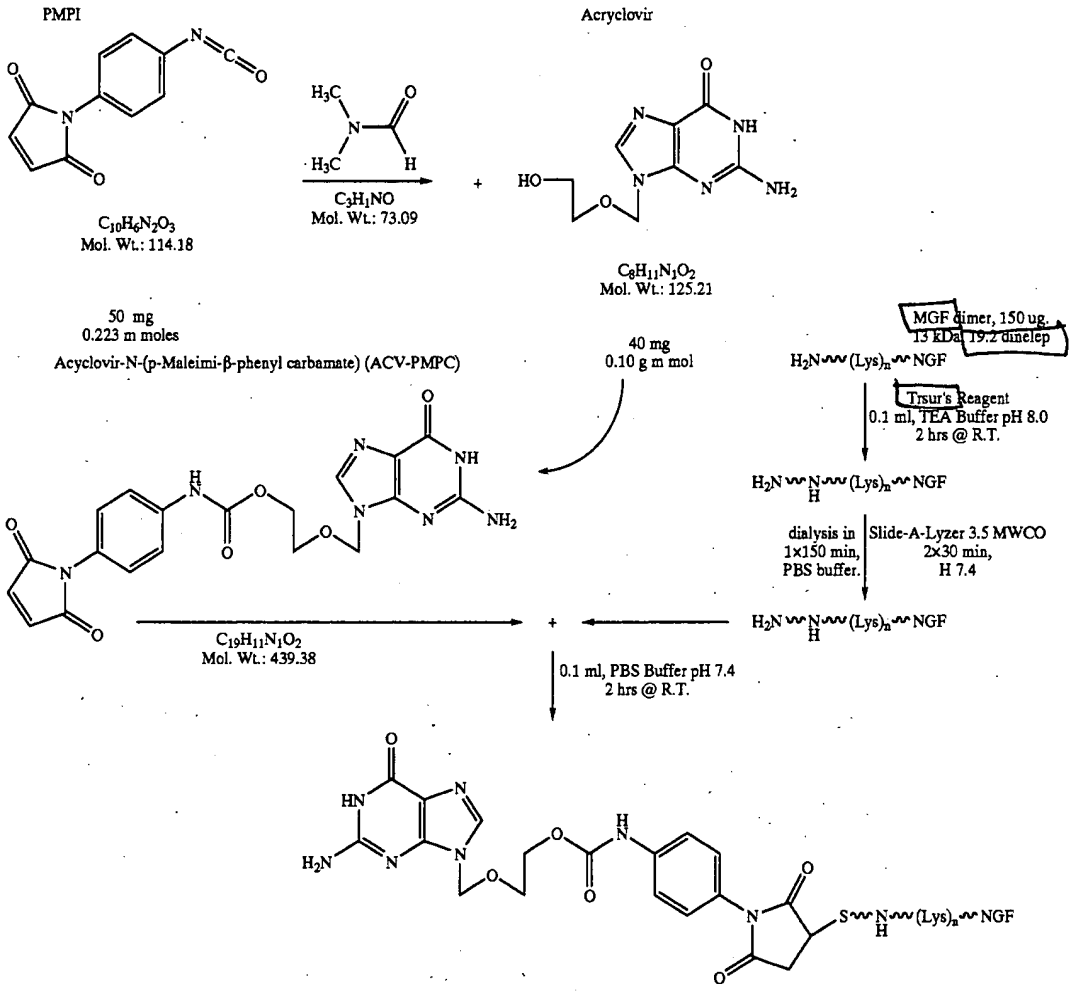
TABLE 4-continued

Phosphate group conjugations

Imidazolide Linker
acyclovir-monophoshate (ACV-MP)

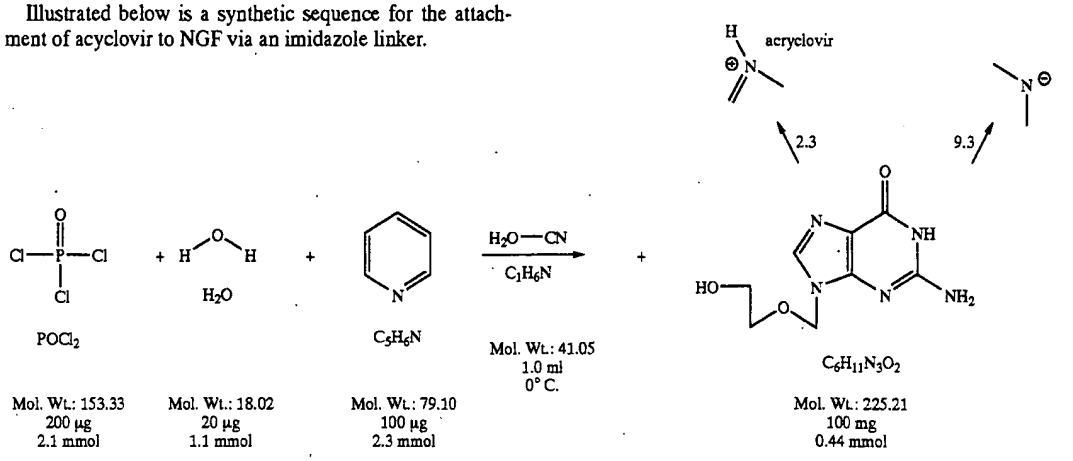
8. Synthetic Sequence for Attaching Acyclovir to NGF Via PMPI

Illustrated below is a synthetic sequence for the attachment of acyclovir to NGF via the linker PMPI.

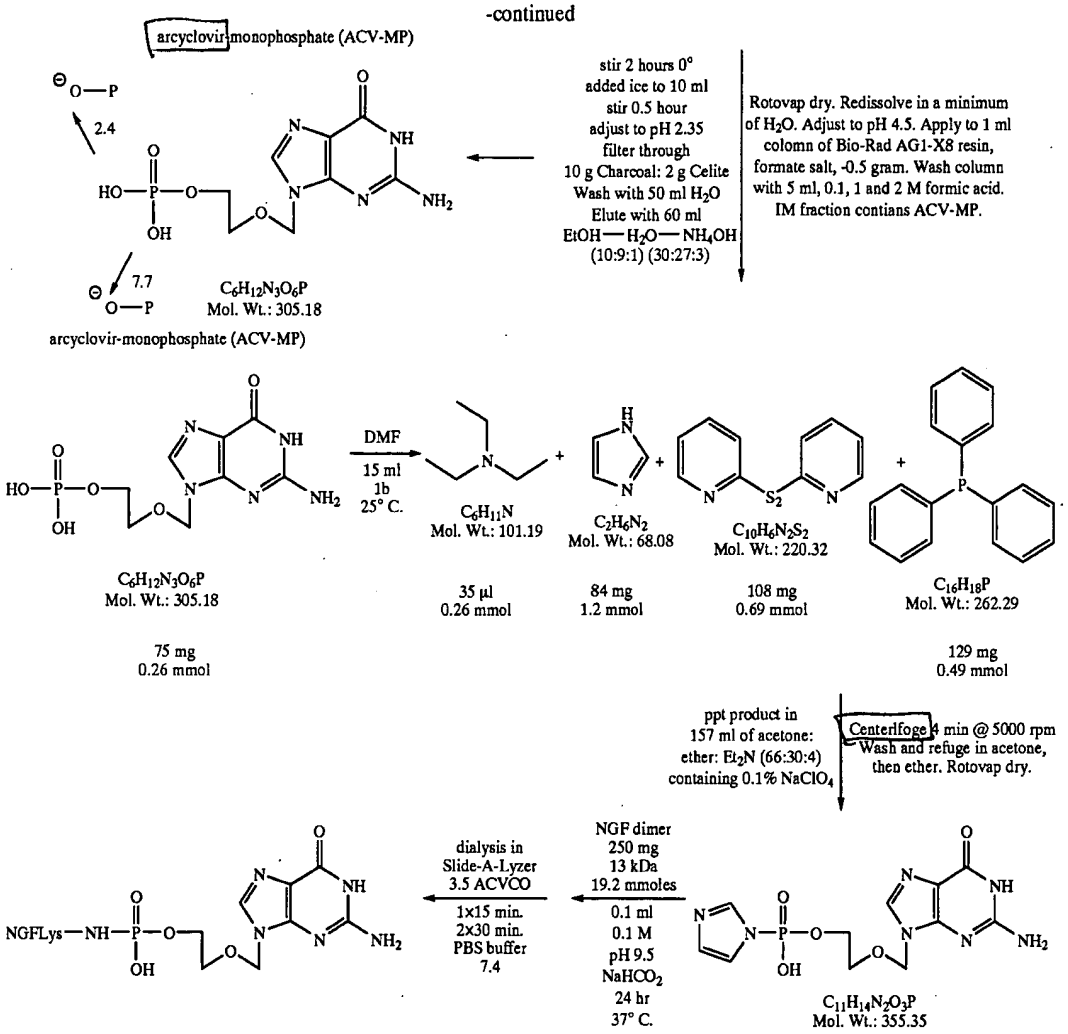


9. Synth Tic Sequence for Attaching Acyclovir to NGF Via Imidazole

Illustrated below is a synthetic sequence for the attachment of acyclovir to NGF via an imidazole linker.



-continued



10. Examples of Human Neurotrophins as the Binding Agent (B)

Table 5 lists the amino acid sequences of human neurotrophins (NGF, BDNF, NT-3, and NT-4) that are used as the binding agent (B) of the present invention. Lysine residues that may be used to attach to the linker (L) which in turn is conjugated with the therapeutic moiety (TM) are highlighted and underlined in Table 5.

TABLE 5

Sequences of Examples of Human Neurotrophins

NERVE GROWTH FACTOR (NGF) [SEQ ID NO: 1]:

1 SER SER SER HIS PRO ILE PHE HIS ARG GLY GLU PHE SER

VAL CYS ASP SER VAL SER VAL TRP VAL GLY ASP LYS THR

THR ALA THR ASP ILE LYS GLY LYS GLU VAL MET VAL LEU

GLY GLU VAL ASN ILE ASN ASN SER VAL PHE LYS GLN TYR

PHE PHE GLU THR LYS CYS ARG ASP PRO ASN PRO VAL ASP

SER GLY CYS ARG GLY ILE ASP SER LYS HIS TRP ASN SER

TYR CYS THR THR THR HIS THR PHE VAL LYS ALA LEU THR

What is claimed is:

1. A conjugated 1,4-pregnadiene-21-hydroxy steroid wherein a conjugate group pends from a steroid 21 hydroxyl group, and wherein the conjugate group comprises a nerve growth factor (NGF) or NGF receptor-binding fragment thereof.

2. The conjugated steroid of claim 1, having a 21-carbamate linkage to the conjugate group.

3. The conjugated steroid of claim 1, having a 21-phosphoramide linkage to the conjugate group.

4. The conjugated steroid of any one of claims 1–3, herein the NGF or NGF receptor-binding fragment pends covalently through an epsilon amino group of a lysine residue.

5. The conjugated steroid of claim 2, wherein the NGF or NGF receptor binding fragment pends covalently through an epsilon amino group of a thiolated lysine residue.

6. The conjugated steroid of claim 1, wherein the steroid is a corticosteroid.

7. The conjugated steroid of claim 6, wherein the corticosteroid is triamcinolone acetonide.

8. The conjugated steroid of claim 6, wherein the corticosteroid is fluocinolone acetonide.

9. The conjugated steroid of claim 1, wherein the conjugate group comprises NGF.

10. The conjugated steroid of claim 1, wherein the conjugate group comprises a nerve growth factor (NGF) fragment which binds trkA receptor and capable of being internalized therewith.

11. The conjugated steroid of claim 7, in which triamcinolone acetonide is conjugated by a 21-carbamate linkage to nerve growth factor (NGF), or to a receptor-binding fragment of NGF, which pends covalently through an epsilon amino group of a lysine residue.

12. The conjugated steroid of claim 8, in which fluocinolone acetonide is conjugated by a 21-carbamate linkage to nerve growth factor (NGF) or a receptor-binding fragment of NGF, which pends covalently through an epsilon amino group of a lysine residue.

13. The conjugated steroid of claim 6, wherein the corticosteroid is betamethasone.

14. The conjugated steroid of claim 6, wherein the corticosteroid is dexamethasone.

15. The conjugated steroid of claim 13, in which betamethasone is conjugated by a 21-phosphoramide linkage to nerve growth factor (NGF) or a receptor-binding fragment of NGF, which pends covalently through an epsilon amino group of a lysine residue.

16. The conjugated steroid of claim 14, in which dexamethasone is conjugated by a 21-phosphoramide linkage to nerve growth factor (NGF) or a receptor-binding fragment of NGF, which pends covalently through an epsilon amino group of a lysine residue.

17. The conjugated steroid of claim 13, in which betamethasone is conjugated by a 21-carbamate linkage to nerve growth factor (NGF) or a receptor-binding fragment of NGF, which pends covalently through an epsilon amino group of a lysine residue.

18. The conjugated steroid of claim 14, in which dexamethasone is conjugated by a 21-carbamate linkage to nerve growth factor (NGF) or a receptor-binding fragment of NGF, which pends covalently through an epsilon amino group of a lysine residue.

19. The conjugated steroid of claim 9, herein the steroid is a corticosteroid.

20. The conjugated steroid of claim 19, herein the corticosteroid is triamcinolone acetonide.

21. The conjugated steroid of claim 20, in which triamcinolone acetonide is conjugated by a 21-carbamate linkage to the NGF, which pends covalently through an epsilon amino group of a lysine residue.

22. The conjugated steroid of claim 19, wherein the corticosteroid is fluocinolone acetonide.

23. The conjugated steroid of claim 22, in which fluocinolone acetonide is conjugated by a 21-carbamate linkage to the NGF, which pends covalently through an epsilon amino group of a lysine residue.

24. The conjugated steroid of claim 19, wherein the corticosteroid is betamethasone.

25. The conjugated steroid of claim 24, in which betamethasone is conjugated by a 21-phosphoramide linkage to the NGF, which pends covalently through an epsilon amino group of a lysine residue.

26. The conjugated steroid of claim 19, wherein the corticosteroid is dexamethasone.

27. The conjugated steroid of claim 26, in which dexamethasone is conjugated by a 21-phosphoramide linkage to the NGF, which pends covalently through an epsilon amino group of a lysine residue.

28. A conjugated 4-pregnene-21-hydroxy-steroid wherein a conjugate group pends from a steroid 21 hydroxyl group, and wherein the conjugate group comprises a nerve growth factor (NGF) or NGF receptor-binding fragment thereof.

29. The conjugated steroid of claim 28, having a 21-carbamate linkage to the conjugate group.

30. The conjugated steroid of claim 28, having a 21-phosphoramide linkage to the conjugate group.

31. The conjugated steroid of any one of claims 28–30, herein the NGF or NGF receptor-binding fragment pends covalently through an epsilon amino group of a lysine residue.

32. The conjugated steroid of claim 29, wherein the NGF or NGF receptor binding fragment pends covalently through an epsilon amino group of a thiolated lysine residue.

33. The conjugated steroid of claim 28, herein the steroid is a corticosteroid.

34. The conjugated steroid of claim 33, herein the corticosteroid is cortisone.

35. The conjugated steroid of claim 34, in which cortisone is conjugated by a 21-carbamate linkage to nerve growth factor (NGF), or to a receptor-binding fragment of NGF, which pends covalently through an epsilon amino group of a lysine residue.

36. The conjugated steroid of claim 28, wherein the conjugate group comprises a nerve: growth factor (NGF) fragment which binds to trkA receptors and capable of being internalized therewith.

37. The conjugated steroid of claim 28, wherein the conjugate group comprises NGF.

38. The conjugated steroid of claim 37, herein the steroid is a corticosteroid.

39. The conjugated steroid of claim 38, herein the corticosteroid is cortisone.

40. The conjugated steroid of claim 39, in which cortisone is conjugated by a 21-carbamate linkage to the NGF, which pends covalently through an epsilon amino group of a lysine residue.

* * * * *

where:

B is a binding agent capable of selectively binding to a nerve cell surface receptor and mediating absorption of the compound by the nerve cell;

IM is a non-cytotoxic imaging moiety which can be used to image a nerve cell or an intracellular component of the nerve cell; and

L is a linker coupling **B** to **IM**.

In regard to each of the above embodiments, particular classes of binding agents **B** which may be used include, but are not limited to, nucleic acid sequences, peptides, peptidomimetics, antibodies and antibody fragments. Examples of nucleic acids that can serve as the binding agent **B** include, but are not limited to, DNA and RNA ligands that function as antagonists of nerve growth factors or inhibit binding of other growth factors to nerve cell surface receptors. Examples of peptides that can serve as the binding agent **B** include, but are not limited to, members of the nerve growth factors (neurotrophin) family such as NGF, BDNF, NT-3, NT-4, NT-6; derivatives, analogs, and fragments of nerve growth factors such as recombinant molecules of NGF and BDNF; and synthetic peptides that bind to nerve cell surface receptors and have agonist or antagonist activities of nerve growth factors.

Antibodies, derivatives of antibodies and antibody fragments can also serve as the binding agent **B**. Examples of this type of binding agent **B** include, but are not limited to, anti-human trkA monoclonal antibody 5C3 and anti-human p75 monoclonal antibody MC192.

The therapeutic moiety **TM** is selected to perform a non-cytotoxic therapeutic function within nerve cells. Examples of non-cytotoxic functions which the therapeutic moiety **TM** may perform include, but are not limited to, the functions performed by adrenergic agents, analgesics, anti-trauma agents, anti-viral agents, gene therapy agents, and hormones (growth factors, interferons, etc.). Examples of classes of therapeutic moieties include, but are not limited to, adrenergic agents (e.g.,

epinephrine, norepinephrine, dopamine, etenolol, analgesics (e.g., opioids, codeine, oxycodone), anti-trauma agents, anti-viral agents (e.g., acyclovir, gancyclovir, AZT, ddI, ddC, etc.), gene therapy agents (e.g., DNAs or RNAs which introduce a gene or replace a mutated gene), steroids (e.g., cortisone, progesterone, estrogen), and hormones (e.g., growth factors, interferons).

In one particular embodiment, the therapeutic moiety **TM** is a charged moiety. Cells have difficulty transporting charged molecules across cell membranes. According to this embodiment, the binding agent **B** serves to facilitate transport of a charged therapeutic moiety **TM** into a cell. Within the cell, the compound (i.e. the conjugate formed between **B** and **TM**) is metabolized to form a metabolite product that comprises the charged therapeutic moiety **TM**. The metabolite product is less prone to being transported across the cell membrane out of the cell relative to the conjugate because of the metabolism of the conjugate resulting in the separation of the therapeutic moeity **TM** from the binding agent **B**. The metabolite product is also less prone to being transported across the cell membrane out of the cell relative to a non-charged version of the therapeutic moiety due to the charge which the therapeutic moiety carries.

According to this embodiment, compounds are provided which comprise a charged derivative of a therapeutic agent having a therapeutic activity, the charged derivative being conjugated to a protein having a biological activity of being transported across a cell membrane into a cell, the cell metabolizing at least a portion of the protein to form a charged metabolite product that possesses the therapeutic activity of the therapeutic agent, the charged metabolite product being less prone to being transported across the cell membrane out of the cell relative to the conjugate and less prone to being transported across the cell membrane out of the cell relative to the therapeutic agent.

In one particular embodiment, the charged therapeutic moiety **TM** is a quartinery alkyl amine derivative of a therapeutic moiety A particular

example of a quartinery alkyl amine derivative of a therapeutic moiety **TM** is a quartinery alkyl amine of propoxycaine, shown in Table 3.

The imaging moiety **IM** is a non-cytotoxic agent which can be used to locate and optionally visualize a nerve cell or an internal component of the nerve cell which has absorbed the imaging moiety. Fluorescent dyes may be used as an imaging moiety **IM**. Radioactive agents which are non-cytotoxic may also be an imaging moiety **IM**.

In general, the linker may be any moiety which can be used to link the binding agent **B** to the moiety **M**. In one particular embodiment, the linker is a cleavable linker. The use of a cleavable linker enables the moiety **M** linked to the binding agent **B** to be released from the compound once absorbed by the nerve cell. The cleavable linker may be cleaved by a chemical agent, enzymatically, due to a pH change, or by being exposed to energy. Examples of forms of energy which may be used include light, microwave, ultrasound, and radiofrequency.

The present invention also relates to a method for selectively delivering a moiety into nerve cells comprising the steps of:

delivering to a patient a compound having the general formula:

B-L-M

where:

B is a binding agent capable of selectively binding to a nerve cell surface receptor and mediating absorption of the compound by the nerve cell;

M is a moiety which performs a useful non-cytotoxic function when absorbed by a nerve cell; and

L is a linker coupling **B** to **M**.

having the compound selectively bind to a nerve cell surface receptor via the binding agent **B**; and

having the compound be absorbed by the nerve cell mediated by the binding of the binding agent **B** to the nerve cell surface receptor.

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In one embodiment, this method is used in conjunction with the conjugates of the present invention and hence is used in conjunction with the methods of the present invention for selectively delivering a moiety into nerve cells.

In one particular embodiment, the charged therapeutic moiety **TM** is a quartinery alkyl amine derivative of a therapeutic moiety A particular example of a quartinery alkyl amine derivative of a therapeutic moiety **TM** is a quartinery alkyl amine of propoxycaine, shown in Table 3.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to compounds which include a binding agent which binds to a nerve cell surface receptor and facilitates absorption of the compound by the nerve cell; and a moiety. Different Moieties may be included in the compounds of the present invention including therapeutic moieties that are non-cytotoxic to the nerve cells and imaging moieties which can be used to image nerve cells which absorb these compounds.

In one embodiment, compounds of the present invention have the general formula:

B-L-TM

where:

B is a binding agent capable of selectively binding to a nerve cell surface receptor and mediating absorption of the compound by the nerve cell;

TM is a therapeutic moiety which has a non-cytotoxic therapeutic effect when absorbed by a nerve cell; and

L is a linker coupling **B** to **TM**.

According to this embodiment, the binding agent **B** serves as a homing agent for nerve cells by selectively binding to nerve cell surface receptors. The binding agent **B** also serves to facilitate absorption of the compound by the nerve cell. The binding agent **B** can be any molecule which can perform these two functions. Particular classes of binding agents which may be used include, but are not limited to, nucleic acid sequences, peptides, peptidomimetics, antibodies and antibody fragments.

Examples of nucleic acids that can serve as the binding agent **B** include, but are not limited to, DNA and RNA ligands that function as antagonists of nerve growth factors or inhibit binding of other growth

factors to nerve cell surface receptors (Binkley, J., et al., Nucleic Acid Res. 23: 3198-3205 (1995); Jellinek, D., et al., Biochem. 33:10450-10456 (1994)).

Examples of peptides that can serve as the binding agent **B** include, but are not limited to, members of the nerve growth factors (neurotrophin) family such as NGF, BDNF, NT-3, NT-4, NT-6, etc. (see reviews: Frade, J. M., et al., Bioessays 20: 137-145 (1998); Shieh, P. B., Curr. Biol. 7: R627-R630 (1997); Dechant, G., et al., Curr. Opin. Neurobiol. 7: 413-418 (1997); Chao, M. V. and Hempstead, B. L., Trends Neurobiol. 18: 321-326 (1995)); and derivatives, analogs, and fragments of nerve growth factors such as recombinant molecules of NGF and BDNF (Ibanez et al., EMBO J. 10: 2105-2110; Ibanez et al., EMBO J. 12: 2281-2293), synthetic peptides that bind to nerve cell surface receptors and have agonist or antagonist activities of nerve growth factors (Longo, F. M., et al., Cell Regulation 1: 189-195 (1990); LeSauteur, L. et al., J. Biol. Chem. 270: 6564-6569 (1995); Longo F. M., et al., J. Neurosci. Res. 48: 1-17; Longo, et al., Nature Biotech. 14: 1120-1122 (1997)).

Examples of antibodies, derivatives of antibodies and antibody fragments that can serve as the binding agent **B** include, but are not limited to, anti-human trkA monoclonal antibody 5C3 (Kramer, K., et al., Eur. J. Cancer 33: 2020-2091 (1997)), anti-human p75 monoclonal antibody MC192 (Maliatchouk, S. and Saragovi, H. U., J. Neurosci. 17: 6031-7).

According to this embodiment, the therapeutic moiety **TM** is selected to perform a non-cytotoxic therapeutic function within nerve cells. Examples of non-cytotoxic functions which the therapeutic moiety **TM** may perform include, but are not limited to, the functions performed by analgesics, anti-trauma agents, anti-viral agents, gene therapy agents, and hormones (growth factors, interferons, etc.). Examples of classes of therapeutic moieties include, but are not limited to, adrenergic agents (e.g., epinephrine, norepinephrine, dopamine, etenolol), analgesics (e.g., opioids, codeine, oxycodone), anti-trauma agents, anti-viral agents (e.g., acyclovir, gancyclovir, AZT, ddI, ddC, etc.), gene therapy agents (e.g.,

DNAs or RNAs which introduce a gene or replace a mutated gene), steroids (e.g., cortisone, progesterone, estrogen), and hormones (e.g., growth factors, interferons).

The linker **L** serves to link the binding agent **B** to the therapeutic moiety **TM**. A wide variety of linkers are known in the art for linking two molecules together, particularly, for linking a moiety to a peptide or nucleic acid, all of which are included within the scope of the present invention.

Examples of classes of linkers that may be used to link the binding agent **B** to the therapeutic moiety **TM** include amide, alkylamine, thiolether, alkyl, cycloalkyl, aryl linkages such as those described in Hermanson, G.T., Bioconjugate Techniques (1996), Academic Press, San Diego, CA.

In certain applications, it is desirable to release the therapeutic moiety **TM** once the compound has entered the nerve cell, resulting in a release of the therapeutic moiety **TM**. Accordingly, in one variation, the linker **L** is a cleavable linker. This enables the therapeutic moiety **TM** to be released from the compound once absorbed by the nerve cell. This may be desirable when the therapeutic moiety **TM** has a greater therapeutic effect when separated from the binding agent. The therapeutic moiety **TM** may have a better ability to be absorbed by an intracellular component of the nerve cell when separated from the binding agent. Accordingly, it may be necessary or desirable to separate the therapeutic moiety **TM** from the compound so that the therapeutic moiety **TM** can enter the intracellular compartment.

Cleavage of the linker releasing the therapeutic moiety may be as a result of a change in conditions within the nerve cells as compared to outside the nerve cells, for example, due to a change in pH within the nerve cell. Cleavage of the linker may occur due to the presence of an enzyme within the nerve cell which cleaves the linker once the compound enters the nerve cell. Alternatively, cleavage of the linker may occur in response to energy or a chemical being applied to the nerve cell. Examples of types of energies that may be used to effect cleavage of the

linker include, but are not limited to light, ultrasound, microwave and radiofrequency energy.

The linker **L** used to link the binding agent **B** to the therapeutic moiety **TM** may be a photolabile linker. Examples of photolabile linkers include those linkers described in US Patent No. 5,767,288 and No. 4,469,774. The linker **L** used to link the binding agent **B** to the therapeutic moiety **TM** may also be an acid labile linker. Examples of acid labile linkers include linkers formed by using cis-aconitic acid, cis-carboxylic alkatriene, polymaleic anhydride, and other acidlabile linkers, such as those linkers described in US Patent Nos. 5,563,250 and 5,505, 931.

Further examples of cleavable linkers include, but are not limited to the linkers described in Lin, et al., J. Org. Chem. 56:6850-6856 (1991); Ph.D. Thesis of W.-C. Lin, U.C. Riverside, (1990); Hobart, et al., J. Immunological Methods 153: 93-98 (1992) ; Jayabaskaran, et al., Preparative Biochemistry 17(2): 121-141 (1987); Mouton, et al., Archives of Biochemistry and Biophysics 218: 101-108 (1982) ; Funkakoshi, et al., J. of Chromatography 638:21-27 (1993); Gildea, et al., Tetrahedron Letters 31: 7095-7098 (1990); WO 85/04674; and Dynabeads™ (Dynal, Inc., 5 Delaware Drive, Lake Success, NY 11042).

In another embodiment, compounds of the present invention have the general formula:

B-L-IM

where:

B is a binding agent capable of selectively binding to a nerve cell surface receptor and mediating absorption of the compound by the nerve cell;

IM is a non-cytotoxic imaging moiety which can be used to image the nerve cell or an intracellular component of the nerve cell; and

L is a linker coupling **B** to **IM**.

According to this embodiment, the binding agent **B** and linker **L** may be varied as described above with regard to compounds having the general formula **B-L-TM**. Further according to this embodiment, the imaging moiety **IM** may be a non-cytotoxic moiety which can be used to image nerve cells. Examples of imaging moieties that may be used include fluorescent dyes and radioisotopes which are non-cytotoxic.

The present invention also relates to a method for selectively delivering a non-cytotoxic therapeutic moiety into nerve cells comprising the steps of:

delivering to a patient a therapeutic amount of a compound having the general formula:

B-L-TM

where:

B is a binding agent capable of selectively binding to a nerve cell surface receptor and mediating absorption of the compound by the nerve cell,

TM is a therapeutic moiety which has a non-cytotoxic therapeutic effect when absorbed by a nerve cell, and

L is a linker coupling **B** to **TM**;

having the compound selectively bind to a nerve cell surface receptor via the binding agent **B**; and

having the compound be absorbed by the nerve cell mediated by the binding of the binding agent **B** to the nerve cell surface receptor.

The method of the present invention offers the advantage of specifically targeting a non-cytotoxic therapeutic moiety to nerve cells where the therapeutic moiety is absorbed by the nerve cells. The method utilizes the fact that internalization of the conjugated agent is mediated by the binding of the binding agent **B** to nerve cell surface receptors. Once internalized, the therapeutic moiety can accumulate within the nerve cells where it has a therapeutic effect. The ability to selectively deliver the

746 (1990)) with a variety of agents including cytokines, steriods, vitamins, hormones, and unidentified components of serum. Specific examples of agents known to induce NGF include 4-methylcatechol, clenbuterol, isoprenaline, L-tryptophan, 1,25-dihydroxyvitamin D3, forskolin, fellutamide A, gangliosides and quinone derivatives (Riaz, S. S. and Tomlinson, D. R. Prog. Neurobiol. 49: 125-143 (1996)).

The method according to the present invention can also be used to deliver antiviral drugs into nerve cells in order to treat diseases caused by viral infection, to eliminate viruses spread to the nerves, and to inhibit infection by such viruses. Examples of viruses that infect the nervous system include but are not limited to rabies viruses, herpes viruses, polioviruses, arboviruses, reoviruses, pseudorabies, corona viruses, and Borna disease viruses. For example, antiviral drugs such as acyclovir, gancyclovir and Cifodovir can be conjugated to the binding agent and used to inhibit active or latent herpes simplex viruses in the peripheral and central nervous system. Specific delivery of the conjugate containing these antiviral drugs to the nervous system can reduce the side effects associated with high doses or long-term administration of these drugs, such as headaches, rash and paresthesia.

The method according to the present invention can also be used to deliver marker compounds to image intracellular components of the nerve cells. Such marker compounds include but are not limited to fluorescent dyes, radioactive complexes, and other luminophores.

The method according to the present invention can also be used to perform gene therapy wherein nucleic acids (DNA or RNA) are delivered to the nerve cells. These nucleic acids may serve to replace genes which are either defective, absent or otherwise not properly expressed by the patient's nerve cell genome.

The above and other features and advantages of the present invention will become more apparent in the following description of the preferred embodiments in greater detail.

2. Therapeutic Moiety (TM)

An aspect of the present invention relates to the delivery of compounds into nerve cells which are non-cytotoxic to the nerve cells and perform a therapeutic function. Examples of therapeutic functions include, but are not limited to, treatment of neurological disorders, gene therapy, intracellular target imaging, cell sorting, or separation schemes. Examples of classes of therapeutic moieties include, but are not limited to adrenergic agents such as epinephrine, norepinephrine, dopamine, etenolol; analgesics such as opioids, codeine, oxycodone; anti-trauma agents; anti-viral agents such as acyclovir, gancyclovir, AZT, ddI, ddC; gene therapy agents such as; steroids such as cortisone, progesterone, estrogen; and hormones such as growth factors and interferons. Such compounds may optionally also include an imaging moiety, such as fluorescent moieties, for imaging intracellular components of the nerve cells.

A further aspect of the present invention relates to compositions and methods for improving the delivery of a therapeutic agent having a therapeutic activity intracellularly. This is accomplished by using therapeutic moieties which are charged. Cells have difficulty transporting charged molecules across cell membranes. According to this embodiment, the binding agent **B** serves to facilitate transport of a charged therapeutic moiety **TM** into a cell. Within the cell, the compound (i.e. the conjugate formed between **B** and **TM**) is metabolized to form a metabolite product that comprises the charged therapeutic moiety **TM**. The metabolite product is less prone to being transported across the cell membrane out of the cell relative to the conjugate because of the metabolism of the conjugate resulting in the separation of the therapeutic moeity **TM** from the binding agent **B**. The metabolite product is also less prone to being transported across the cell membrane out of the cell relative to a non-charged version of the therapeutic moiety due to the charge which the therapeutic moiety carries.

According to this embodiment, compounds are provided which comprise a charged derivative of a therapeutic agent having a therapeutic activity, the charged derivative being conjugated to a protein having a biological activity of being transported across a cell membrane into a cell, the cell metabolizing at least a portion of the compound to form a charged metabolite product that possesses the therapeutic activity of the therapeutic agent, the charged metabolite product being less prone to being transported across the cell membrane out of the cell relative to the compound and less prone to being transported across the cell membrane out of the cell relative to the therapeutic agent.

In one particular embodiment, the charged therapeutic moiety **TM** is a quartinery alkyl amine derivative of a therapeutic moiety. A particular example of a quartinery alkyl amine derivative of a therapeutic moiety **TM** is a quartinery alkyl amine of propoxycaine, shown in Table 3.

Also according to this embodiment, methods are provided which comprise administering a therapeutic agent to a patient in a form where the therapeutic agent comprises a charge and is conjugated to a protein having the biological activity of being transported across a cell membrane into a cell. Once within the cell, the cell metabolizes at least a portion of the compound to form a metabolite product that possesses the therapeutic activity of the therapeutic agent. The metabolite product is less prone to being transported across the cell membrane out of the cell relative to the compound because of the metabolism of the compound resulting separation of the therapeutic moiety from the protein, and is less prone to being transported across the cell membrane out of the cell relative to an uncharged version of the therapeutic agent.

This method may be used in conjunction with the conjugates of the present invention for selectively delivering a moiety to nerve cells. However, it is noted that charged therapeutic moieties can be used with binding agents that target cells other than nerve cells.

3. Linker (L)

According to the present invention, a binding agent **B** is linked to a therapeutic moiety **TM** by a linker **L**. In general, any method of linking a binding agent to a therapeutic moiety may be used and is intended to fall within the scope of the present invention.

Many different types of linkers have been developed for cross linking proteins and conjugating proteins or peptides with other agents. These linkers include zero-length cross linkers, homobifunctional cross-linkers, heterobifunctional cross-linkers and trifunctional cross-linkers. These linkers may have different susceptibility to cleavage under certain conditions. Depending on a particular application according to the present invention, an appropriate linker may be chosen. When an intracellular release of the agent from its conjugate is desired, a cleavable linker is chosen which is susceptible to cleavage by external stimuli such as light and heat, by intracellular enzymes, or by a particular microenvironment inside the cell.

In one embodiment, the linker **L** has one of the following general structures:

B-R_1-(CO)-NH –R_2-**TM**

B-R_3-NH-R_4-**TM**

B-R_3-S –R_4-**TM**

B-R_5-$(CH_2)_n$ -R_6-**TM**

Wherein R_1, R_2, R_3, R_4, R_5, and R_6 are independently selected from the group consisting of alkyls, aryls, heteroaryls, cycloalkyls, cycloalkenes and heterocycloalkenes.

6. Examples Of Compounds For Treating Pain

Table 3 provides several therapeutic moieties which may be used in the compounds and methods of the present invention for treating pain. It is noted that any of the various binding moieties and linkers described herein may be employed with these therapeutic agents. Indicated in the table below as * are preferred moieties for attaching linkers to the therapeutic moieties.

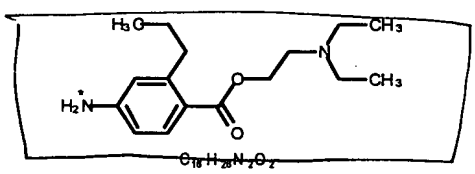
7. Examples Of Linkers

Table 4 provides a series of linkers for linking different therapeutic moieties and binding moieties together. As illustrated, linkers are provided for attaching moieties which have thiol (-SH), hydroxyl (-OH), and amino (-NH2) groups to the linkers. In these examples, neurotrohin is shown as the binding agent. However, it is noted that neurotrohin and these examples are intended to be exemplary only. Other linkers may also be used and are intended as part of the present invention.

TABLE 3 (cont.)

Pain - Local anesthetic agents

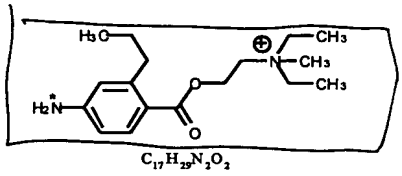
Propoxycaine



$C_{16}H_{26}N_2O_2$

Mol. Wt.: 278.39

Quaternary Propoxycaine Derivative

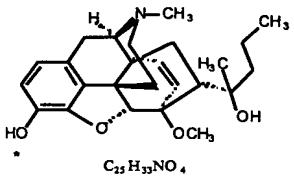


$C_{17}H_{29}N_2O_2$

Mol. Wt.: 293.42

Pain - Narcotic Agonists

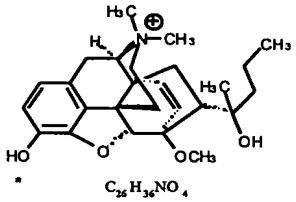
Etorphine



$C_{25}H_{33}NO_4$

Mol. Wt.: 411.54

Quaternary Etorphine Derivative

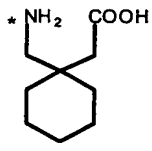


$C_{26}H_{36}NO_4$

Mol. Wt.: 426.57

Pain - Channel blockers

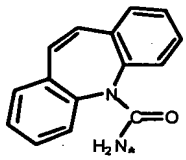
Gabapentin



$C_9H_{17}NO_2$

Mol. Wt.: 171.24

Carbamazepine



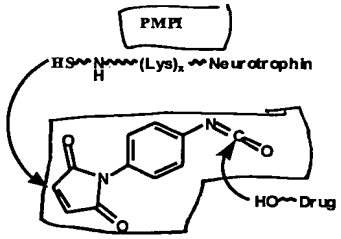
$C_{15}H_{12}N_2O$

Mol. Wt.: 236.27

Table 4

Hydroxyl group conjugations

e.g., Steroids, Piroxicam, Acyclovir, Etorphines

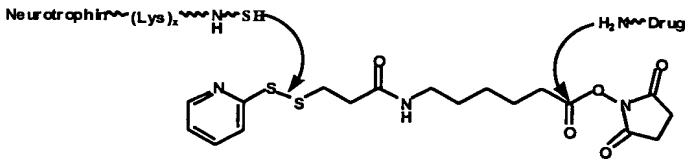


$C_{11}H_6N_2O_3$ Mol. Wt.: 214.18 Spacer Arm = 8.7 Å

Amino group conjugations

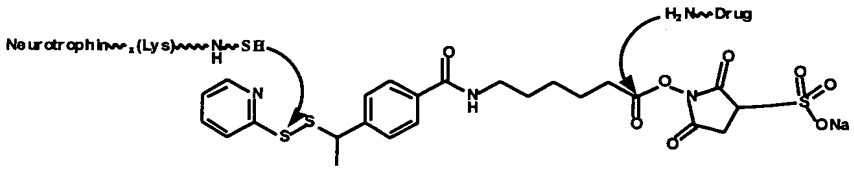
e.g., Propoxycaines, Gabapentin, Carbemazepine, Tacrine

LC-SPDP



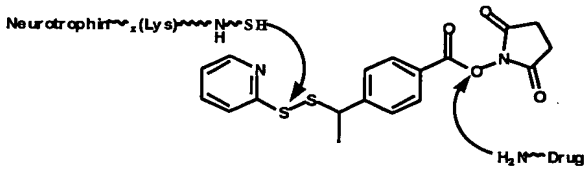
$C_{19}H_{21}N_3O_5S_2$ Mol. Wt.: 425.52 Spacer Arm = 15.7 Å

Sulfo-LC-SMPT



$C_{25}H_{28}N_3NaO_8S_3$ Mol. Wt.: 617.69 Spacer Arm = 20.0 Å

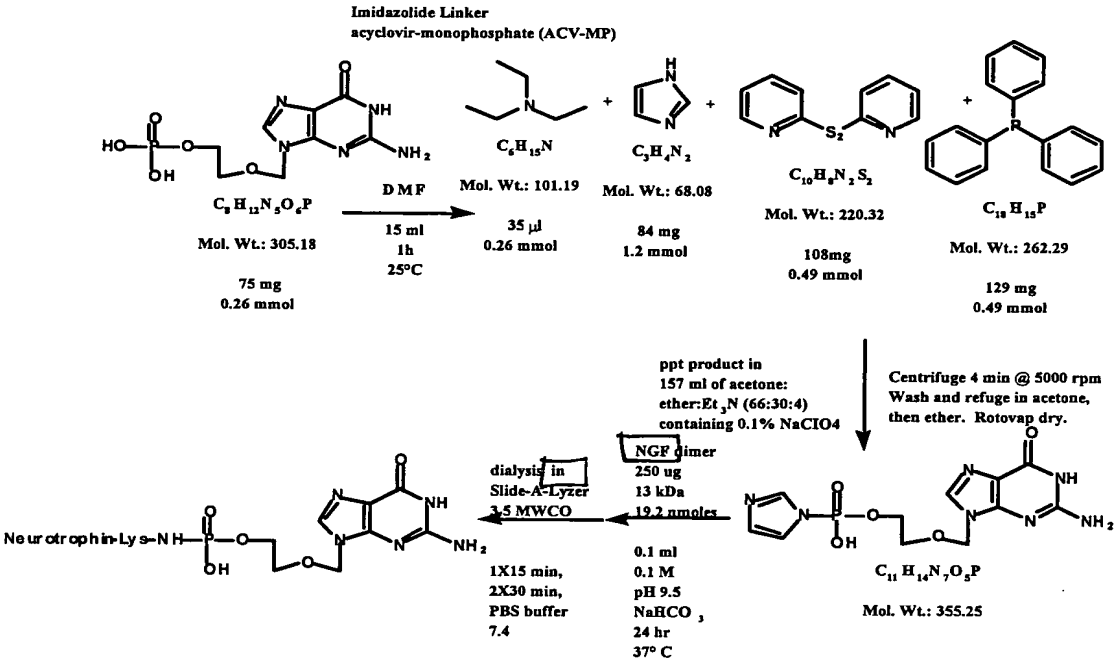
SMPT



$C_{18}H_{16}N_2O_4S_2$ Mol. Wt.: 388.46 Spacer Arm = 11.8 Å

Table 4 (cont.)

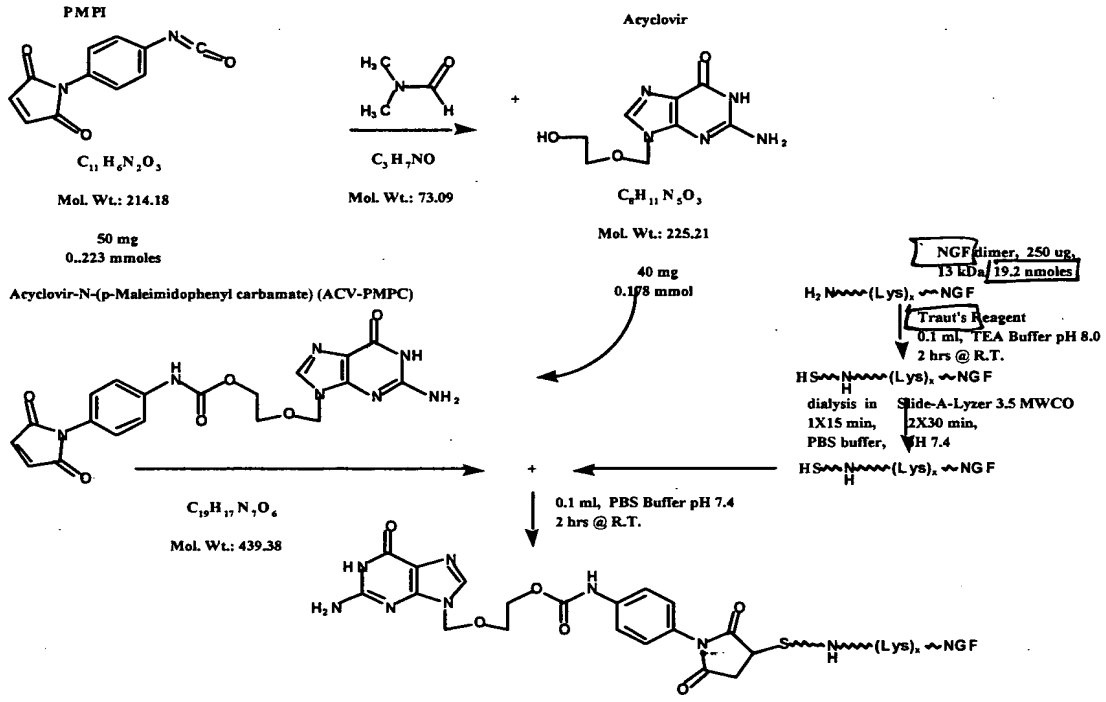
Phospate group conjugations



5

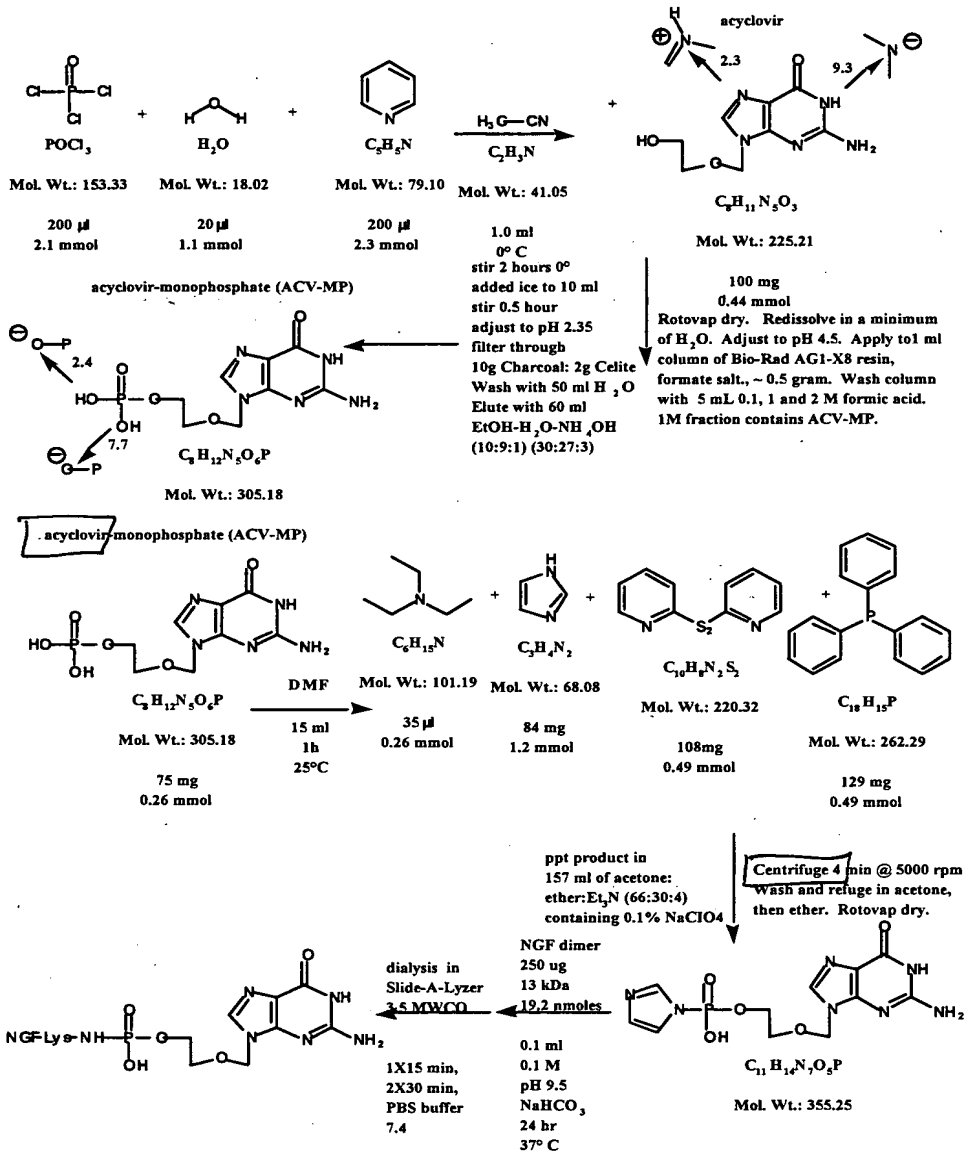
8. Synthetic Sequence For Attaching Acyclovir To NGF Via PMPI

Illustrated below is a synthetic sequence for the attachment of acyclovir to NGF via the linker PMPI.



9. Synthetic Sequence For Attaching Acyclovir To NGF Via Imidazole

Illustrated below is a synthetic sequence for the attachment of acyclovir to NGF via an imidazole linker.



10. Examples of Human Neurotrophins as the Binding Agent (B)

Table 5 lists the amino acid sequences of human neurotrophins (NGF, BDNF, NT-3, and NT-4) that are used as the binding agent (**B**) of the present invention. Lysine residues that may be used to attach to the

COPY

Atty/Sec: CLF/MC
Atty Docket No. ASIL-002CIP
Application No.: 09/707,730
Confirmation No.: 8812
Inventor(s): HILL, CRAIG
Title: "COMPOUNDS FOR INTRACELLULAR DELIVERY OF THERAPEUTIC MOIETIES TO NERVE CELLS"

Date Mailed: September 28, 2004
Filing Date: November 6, 2000

Enclosure(s):

- Transmittal (1 pg.)
- Fee Transmittal in Duplicate (2 pgs.)
- Amendment (12 pgs.)
- Supplemental Information Disclosure Statement (2 pgs.)
- PTO/SB/08 form (3 pgs.)
- 22 References
- Copy of Information Disclosure Statement filed 5-20-03 (3 pgs) including 3 references

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TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Total Number of Pages in This Submission: 23

Application Number	09/707,730
Filing Date	November 6, 2000
First Named Inventor	HILL, G. CRAIG
Group Art Unit	1647
Examiner Name	LANDSMAN, ROBERT S.
Attorney Docket Number	ASIL-002CIP

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- [X] Amendment / Reply
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- [X] Information Disclosure Statement
  - SB08a form
- [ ] Certified Copy of Priority Documents
- [ ] Response to Missing Parts/ Incomplete Application
  - [ ] Response to Missing Parts under 37 CFR 1.52 or 1.53
- [ ] Assignment Papers *(for an Application)*
- [ ] Drawing(s)
- [ ] Licensing-related Papers
- [ ] Petition
- [ ] Petition to Convert to a Provisional Application
- [ ] Power of Attorney, Revocation Change of Correspondence Address
- [ ] Terminal Disclaimer
- [ ] Request for Refund
- [ ] CD, Number of CD(s
- [ ] After Allowance Communication to Group
- [ ] Appeal Communication to Board of Appeals and Interferences
- [ ] Appeal Communication to Group *(Appeal Notice, Brief, Reply Brief)*
- [ ] Proprietary Information
- [ ] Status Letter
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  - *22 References*
  - *Copy of IDS filed on 5/20/03 including 3 references*
  - *Postcard*

Remarks

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Signing Attorney/Agent (Reg. No.)	CAROL L. FRANCIS, 36,513 BOZICEVIC, FIELD & FRANCIS, LLP
Signature	
Date	September 28, 2004

09/28/04 DOCKETED

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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

FEE TRANSMITTAL for FY 2004

Effective 10/01/2003. Patent fees are subject to annual revision.

☒ Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT **($) 180.00**

Complete if Known	
Application Number	09/707,730
Filing Date	November 6, 2000
First Named Inventor	HILL, G. CRAIG
Examiner Name	LANDSMAN, ROBERT S.
Art Unit	1647
Attorney Docket No.	ASIL-002CIP

METHOD OF PAYMENT (check all that apply)

☐ Check ☐ Credit Card ☐ Money Order ☐ Other ☐ None

☒ Deposit Account:

Deposit Account Number: 50-0815

Deposit Account Name: Bozicevic, Field & Francis, LLP

The Director is authorized to: (*check all that apply*)

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FEE CALCULATION

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1001	770	2001	385	Utility filing fee	
1002	340	2002	170	Design filing fee	
1003	530	2003	265	Plant filing fee	
1004	770	2004	385	Reissue filing fee	
1005	160	2005	80	Provisional filing fee	

SUBTOTAL (1)

2. EXTRA CLAIM FEES FOR UTILITY AND REISSUE

	Extra Claims	Fee from below	Fee Paid
Total Claims	-20** =	x	=
Indep. Claims	-3** =	x	=
Multiple Dependent			=

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
1202	18	2202	9	Claims in excess of 20
1201	86	2201	43	Independent claims in excess of 3
1203	290	2203	145	Multiple dependent claim, if not paid
1204	86	2204	43	** Reissue independent claims over original patent
1205	18	2205	9	** Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) $

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FEE CALCULATION (continued)

3. ADDITIONAL FEES

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1051	130	2051	65	Surcharge – late filing fee or oath	
1052	50	2052	25	Surcharge – late provisional filing fee or cover sheet	
1053	130	1053	130	Non-English specification	
1812	2,520	1812	2,520	For filing a request for *ex parte* reexamination	
1804	920*	1804	920*	Requesting publication of SIR prior to Examination action	
1805	1,840*	1805	1,840*	Requesting publication of SIR after Examiner action	
1251	110	2251	55	Extension for reply within first month	
1252	420	2252	210	Extension for reply within second month	
1253	950	2253	475	Extension for reply within third month	
1254	1,480	2254	740	Extension for reply within fourth month	
1255	2,010	2255	1,005	Extension for reply within fifth month	
1401	330	2401	165	Notice of Appeal	
1402	330	2402	165	Filing a brief in support of an appeal	
1403	290	2403	145	Request for oral hearing	
1451	1,510	1451	1,510	Petition to institute a public use proceeding	
1452	110	2452	55	Petition to revive – unavoidable	
1453	1,330	2453	665	Petition to revive – unintentional	
1501	1,330	2501	665	Utility issue fee (or reissue)	
1502	480	2502	240	Design issue fee	
1503	640	2503	320	Plant issue fee	
1460	130	1460	130	Petitions to the Commissioner	
1807	50	1807	50	Processing fee under 37 CFR 1.17(q)	
1806	180	1806	180	Submission of Information Disclosure Stmt	180.00
8021	40	8021	40	Recording each patent assignment per property (times number of properties)	
1809	770	2809	385	Filing a submission after final rejection (37 CFR § 1.129(a))	
1810	770	2810	385	For each additional invention to be examined (37 CFR § 1.129(b))	
1801	770	2801	385	Request for Continued Examination (RCE)	
1802	900	1802	900	Request for expedited examination of a design application	

Other fee (specify) _____

*Reduced by Basic Filing Fee Paid **SUBTOTAL (3) ($)** 180.00

SUBMITTED BY		Complete (*if applicable*)			
Name (Print/Type)	Carol A. Francis	Registration No. (Attorney/Agent)	36,513	Telephone	(650) 833-7713
Signature				Date	09/28/2004

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FEE TRANSMITTAL for FY 2004

Effective 10/01/2003. Patent fees are subject to annual revision.

☒ Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT **($) 180.00**

Complete if Known	
Application Number	09/707,730
Filing Date	November 6, 2000
First Named Inventor	HILL, G. CRAIG
Examiner Name	LANDSMAN, ROBERT S.
Art Unit	1647
Attorney Docket No.	ASIL-002CIP

METHOD OF PAYMENT (check all that apply)

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☒ Deposit Account:

Deposit Account Number: 50-0815

Deposit Account Name: Bozicevic, Field & Francis, LLP

The Director is authorized to: (*check all that apply*)

☒ Charge fee(s) indicated below ☒ Credit any overpayments

☒ Charge Any Additional Fee(s) Required under 37 C.F.R. 1.17.

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2. EXTRA CLAIM FEES FOR UTILITY AND REISSUE

	Extra Claims		Fee from below	Fee Paid
Total Claims	-20** =		x	=
Indep. Claims	-3** =		x	=
Multiple Dependent				=

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
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SUBTOTAL (2) $

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FEE CALCULATION (continued)

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1453	1,330	2453	665	Petition to revive – unintentional	
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1802	900	1802	900	Request for expedited examination of a design application	
				Other fee (specify) _____	

*Reduced by Basic Filing Fee Paid **SUBTOTAL (3) ($)** 180.00

SUBMITTED BY		Complete (*if applicable*)			
Name (Print/Type)	Carol A. Francis	Registration No. (Attorney/Agent)	36,513	Telephone	(650) 833-7713
Signature				Date	09/28/2004

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AMENDMENT UNDER 37 C.F.R. §1.111	Attorney Docket No.	ASIL-002CIP
	Confirmation No.	8812
	First Named Inventor	HILL, G. CRAIG
	Application Number	09/707,730
Address to: Mail Stop Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450	Filing Date	November 6, 2000
	Group Art Unit	1647
	Examiner Name	LANDSMAN, ROBERT S.
	Title: "COMPOUNDS FOR INTRACELLULAR DELIVERY OF THERAPEUTIC MOIETIES TO NERVE CELLS"	

Sir:

This amendment is responsive to the Office Action dated June 28, 2004 for which a three-month period for response was given making this response due on or before September 28, 2004. Accordingly, this response is timely filed. In view of the amendments to the claims and the remarks put forth below, reconsideration and allowance are respectfully requested.

COPY

AMENDMENTS TO THE CLAIMS:

1.-24. **(Canceled)**

25. **(Currently Amended)** A conjugated ~~4-pregnen-21-hydroxy-or~~ 1,4-pregnadiene-21-hydroxy steroid wherein ~~the~~ **a conjugate** ~~conjugant~~ group pends from ~~the steroid~~ **a steroid** 21 hydroxyl group, ~~which conjugant~~ **and wherein the conjugate** group comprises a nerve growth factor (NGF) or NGF receptor-binding fragment thereof.

26. **(Currently Amended)** The conjugated steroid of claim 25, having a 21-carbamate linkage to the **conjugate** ~~conjugant~~ group.

27. **(Currently Amended)** The conjugated steroid of claim 25, having a 21-phosphoramide linkage to the **conjugate** ~~conjugant~~ group.

28. **(Currently Amended)** The conjugated steroid of any one of claims 25 - 27, wherein the NGF or NGF receptor-binding fragment pends covalently through **an epsilon amino group of** a lysine residue ~~epsilon amino group~~.

29. **(Currently Amended)** The conjugated steroid of claim 26, wherein the NGF or NGF receptor binding fragment pends covalently through **an epsilon amino group of** a thiolated lysine residue ~~epsilon amino group~~.

30. **(Currently Amended)** The conjugated steroid of **claim 25** ~~claim 28~~, wherein the steroid is a corticosteroid.

31. **(Canceled)**

32. **(Currently Amended)** The conjugated steroid of claim 30, wherein the ~~steroid~~ **corticosteroid** is triamcinolone acetonide.

SEP 28 2005

SEP 28 2005

33. **(Currently Amended)** The conjugated steroid of claim 30, wherein the ~~steroid~~ **corticosteroid** is fluocinolone acetonide.

34. **(Canceled)**

35. **(Currently Amended)** The conjugated steroid of ~~claim 28~~ **claim 25**, wherein the **conjugate** ~~conjugant~~ group comprises NGF ~~(NGF)~~.

36. **(Currently Amended)** The conjugated steroid of ~~claim 35~~ **claim 25**, wherein the **conjugate** ~~conjugant~~ group comprises a nerve growth factor (NGF) fragment **which binds** ~~capable of binding~~ to trkA receptors and being internalized therewith.

37. **(Canceled)**

38. **(Canceled)**

39. **(Currently Amended)** The conjugated steroid of ~~claim 25~~ **claim 32**, in which triamcinolone acetonide is conjugated by a 21-carbamate linkage to nerve growth factor (NGF), or to a receptor-binding fragment of NGF, which pends covalently through **an epsilon amino group of** a lysine residue ~~epsilon amino group~~.

40. **(Currently Amended)** The conjugated steroid of ~~claim 25~~ **claim 33**, in which fluocinolone acetonide is conjugated by a 21-carbamate linkage to nerve growth factor (NGF) or a receptor-binding fragment of NGF, which pends covalently through **an epsilon amino group of** a lysine residue ~~epsilon amino group~~.

41. **(Currently Amended)** The conjugated steroid of claim 30, wherein the ~~steroid~~ **corticosteroid** is betamethasone.

42. **(Currently Amended)** The conjugated steroid of claim 30, wherein the ~~steroid~~ **corticosteroid** is dexamethasone.

43. **(Currently Amended)** The conjugated steroid of ~~claim 25~~ **claim 41**, in which betamethasone is conjugated by a 21-phosphoramide linkage to nerve growth factor (NGF) or a receptor-binding fragment of NGF, which pends covalently through **an epsilon amino group of** a lysine residue ~~epsilon amino group~~.

44. **(Currently Amended)** The conjugated steroid of ~~claim 25~~ **claim 42**, in which dexamethasone is conjugated by a 21-phosphoramide linkage to nerve growth factor (NGF) or a receptor-binding fragment of NGF, which pends covalently through **an epsilon amino group of** a lysine residue ~~epsilon amino group~~.

45. **(Canceled)**

46. **(Canceled)**

47. **(New)** The conjugated steroid of claim 41, in which betamethasone is conjugated by a 21-carbamate linkage to nerve growth factor (NGF) or a receptor-binding fragment of NGF, which pends covalently through an epsilon amino group of a lysine residue.

48. **(New)** The conjugated steroid of claim 42, in which dexamethasone is conjugated by a 21-carbamate linkage to nerve growth factor (NGF) or a receptor-binding fragment of NGF, which pends covalently through an epsilon amino group of a lysine residue.

49. **(New)** The conjugated steroid of claim 35, wherein the steroid is a corticosteroid.

50. **(New)** The conjugated steroid of claim 49, wherein the corticosteroid is triamcinolone acetonide.

51. **(New)** The conjugated steroid of claim 50, in which triamcinolone acetonide is conjugated by a 21-carbamate linkage to the NGF, which pends covalently through an epsilon amino group of a lysine residue.

52. **(New)** The conjugated steroid of claim 49, wherein the corticosteroid is fluocinolone acetonide.

53. **(New)** The conjugated steroid of claim 52, in which fluocinolone acetonide is conjugated by a 21-carbamate linkage to the NGF, which pends covalently through an epsilon amino group of a lysine residue

54. **(New)** The conjugated steroid of claim 49, wherein the corticosteroid is betamethasone.

55. **(New)** The conjugated steroid of claim 54, in which betamethasone is conjugated by a 21-phosphoramide linkage to the NGF, which pends covalently through an epsilon amino group of a lysine residue.

56. **(New)** The conjugated steroid of claim 49, wherein the corticosteroid is dexamethasone.

57. **(New)** The conjugated steroid of claim 56, in which dexamethasone is conjugated by a 21-phosphoramide linkage to the NGF, which pends covalently through an epsilon amino group of a lysine residue.

58. **(New)** A conjugated 4-pregnene-21-hydroxy-steroid wherein a conjugate group pends from a steroid 21 hydroxyl group, and wherein the conjugate group comprises a nerve growth factor (NGF) or NGF receptor-binding fragment thereof.

59. **(New)** The conjugated steroid of claim 58, having a 21-carbamate linkage to the conjugate group.

60. **(New)** The conjugated steroid of claim 58, having a 21-phosphoramide linkage to the conjugate group.

61. (New) The conjugated steroid of any one of claims 58 - 60, wherein the NGF or NGF receptor-binding fragment pends covalently through an epsilon amino group of a lysine residue.

62. (New) The conjugated steroid of claim 59, wherein the NGF or NGF receptor binding fragment pends covalently through an epsilon amino group of a thiolated lysine residue.

63. (New) The conjugated steroid of claim 58, wherein the steroid is a corticosteroid.

64. (New) The conjugated steroid of claim 63, wherein the corticosteroid is cortisone.

65. (New) The conjugated steroid of claim 64, in which cortisone is conjugated by a 21-carbamate linkage to nerve growth factor (NGF), or to a receptor-binding fragment of NGF, which pends covalently through an epsilon amino group of a lysine residue.

66. (New) The conjugated steroid of claim 58, wherein the conjugate group comprises a nerve growth factor (NGF) fragment which binds to trkA receptors and being internalized therewith.

67. (New) The conjugated steroid of claim 58, wherein the conjugate group comprises NGF.

68. (New) The conjugated steroid of claim 63, wherein the steroid is a corticosteroid.

69. (New) The conjugated steroid of claim 68, wherein the corticosteroid is cortisone.

70. (New) The conjugated steroid of claim 69, in which cortisone is conjugated by a 21-carbamate linkage to the NGF, which pends covalently through an epsilon amino group of a lysine residue.

REMARKS

FORMAL MATTERS:

Claims 25-30, 32-33, 35-36, 39-44, and 47-70 are pending after entry of the amendments set forth herein.

Claims 25-30, 31-33, 35-36, and 39-46 have been objected to.

Claims 31, 37, and 38 have been canceled. Claims 25-30, 32-33, 35-36, 39-44 are amended to correct typographical and dependency errors.

New Claims 47-70 have been added. Support for new Claims 47-70 is found throughout the specification and in the claims as originally filed, for example:

Claim	Support
47	Claims 25, 26, and 41
48	Claims 25, 26, and 42
49	Claim 30
50	Claim 32
51	Claim 39
52	Claim 33
53	Claim 40
54	Claim 41
55	Claim 43
56	Claim 42
57	Claim 44
58	Claim 25
59	Claim 26
60	Claim 27
61	Claim 28
62	Claim 29
63	Claim 30
64	Claim 45
65	Claim 46

Claim	Support
66	Claim 36
67	Claim 35
68	Claim 30
69	Claim 45
70	Claim 46

Accordingly, no new matter has been added.

INFORMATION DISCLOSURE STATEMENT:

IDS Originally Filed May 20, 2003

Applicants hereby resubmit with this communication the IDS originally filed on May 20, 2003. Applicants respectfully request that the Examiner consider the references cited in the Information Disclosure Statement, originally filed on May 20, 2003, and indicate such consideration by initialing the PTO from SB/08A and returning a copy of the initialed form with the next Action.

New IDS Filed Herewith

Applicants respectfully request that the Examiner consider the references cited in the new Information Disclosure Statement (IDS) filed with this communication, and indicate such consideration by initialing the PTO form SB/08A and returning a copy of the initialed form with the Action. The Applicants note that the references cited in the IDS consist of the references cited in the document entitled "Art of Interest" cited in the Office Action dated October 21, 2003.

NEW CLAIMS 47-57

New Claims 47-57 have been added, which new claims find support in the claims as originally filed as noted above. Accordingly, new Claims 47-57 do not present new matter that would require further examination. New Claims 47-57 incorporate the amendments made to Claims 30, 32, 33, 39-44 in order to overcome objections set out in the Office Action dated June 28, 2004. As such, new Claims 47-57 should be free of similar objections.

NEW CLAIMS 58-70

Claim 25 has been amended to remove the "or 4-pregnene-21-hydroxy" class of compounds and new Claims 58-70 have been added and are directed to 4-pregnene-21-hydroxy containing compounds. Accordingly, new Claims 58-70 do not present new matter that would require further examination. Moreover, new Claims 58-70 also incorporate the amendments made to Claims 25-30, 32, 33, 35, 36, and 39-44 in order to overcome objections set out in the Office Action dated June 28, 2004. As such, new Claims 58-70 should be free of similar objections.

REJECTION OF OFFICE ACTION DATED OCTOBER 21, 2003

The Applicants acknowledge with gratitude the Examiner's indication that the rejection under 35 U.S.C. § 112, first paragraph, as set forth in the Office Action dated October 21, 2003 has been withdrawn.

CLAIM OBJECTIONS

Item A (Office Action page 2)

Claims 25-46 were objected to for use of the term "conjugant". In view of the amendments to the claims, which amendments replace the term "conjugant" with the term "conjugate", this objection may be withdrawn.

Item B (Office Action page 2)

Claims 25-46 were objected to for reasons relating to syntax because the phrase "which conjugate group" was unclear. In view of the amendments to the claims, which amendments replace the phrase "which conjugate group" with the phrase "and wherein said conjugate group", this objection may be withdrawn.

Item C (Office Action page 2)

Claims 28-46 were objected to for reasons relating to syntax. Claims 28, 29, 39, 40, 43, 44, and 46 have been amended to recite "through an epsilon amino group of a lysine residue". Accordingly, this objection may be withdrawn.

Item D (Office Action page 2)

Claims 32 and 33 were objected to because it allegedly appeared that the claims should depend from claim 31. Claim 31 has been canceled and claims 32 and 33 have been amended to depend from 30. In view of the amendments to the claims, this objection may be withdrawn.

Item E (Office Action page 3)

Claim 35 was objected to for typographical reasons because the term "NGF" was repeated twice. In view of the amendments to the claim were the second "NGF" was removed, this objection may be withdrawn.

Item F (Office Action page 3)

Claim 36 was objected to for reasons relating dependency. Claim 26 has been amended to depend from Claim 25. Accordingly, this rejection may be withdrawn.

Item G (Office Action page 3)

Claim 36 was objected to for reasons relating to syntax. Claim 36 has been amended to replace the phrase "capable of binding" with the phrase "which binds". Accordingly, this objection may be withdrawn.

Item H (Office Action page 3)

Claims 39 and 40 were objected to for reasons relating dependency. Claim 39 has been amended to depend from Claim 32, and Claim 40 has been amended to depend from Claim 33. Accordingly, this rejection may be withdrawn.

Item I (Office Action page 3)

Claims 41 and 42 were objected to for reasons relating dependency. Claims 41 and 42 have been amended to recite "the **corticosteroid**". Accordingly, this rejection may be withdrawn.

Item J (Office Action page 3)

Claim 43 was objected to for reasons relating to dependency. Claim 43 has been amended to depend from Claim 41. Accordingly, this rejection may be withdrawn.

Item K (Office Action page 3)

Claim 44 was objected to for reasons relating to dependency. Claim 44 has been amended to depend from Claim 42. Accordingly, this rejection may be withdrawn.

Item L (Office Action page 3)

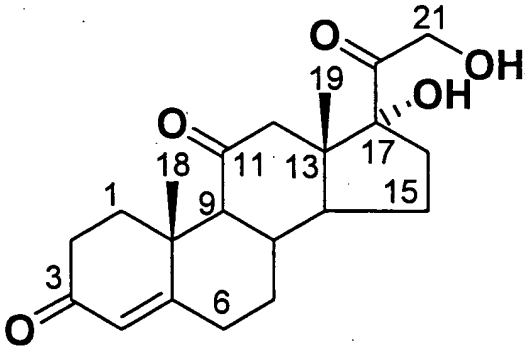
Claim 45 was objected to for reasons relating to dependency. Claim 45 has been canceled, rendering this rejection moot.

Item M (Office Action page 3)

Claim 46 was objected to for reasons relating to dependency. Claim 46 has been canceled, rendering this rejection moot.

REJECTIONS UNDER §112, ¶2

Claim 31 has been rejected under 35 U.S.C. § 112, second paragraph because there allegedly is insufficient antecedent basis for the limitation of "cortisone" recited in the original claim 25. The Office Action notes that it is unclear if cortisone meets the structural requirements recited in the original claim 25. Claim 31 has been canceled rendering this rejection moot. Claim 58 has been added to split the original claim 25 into separate classes of 1,4-pregnadiene-21-hydroxy steroids (claim 25) and 4-pregnene-21-hdyroxy steroids (claim 58). With respect to new claim 64, which depends from new claim 58 and recites cortisone, the applicants note that the structure of Cortisone (17,21-dihydroxypregn-4-ene-3,11,20-trione) is as follows:



Accordingly, the structure of cortisone, which well known in the art, meets the structural requirements recited in original claim 25 and in new claim 58, the latter of which recites the 4-pregnene-21-hydroxy steroid.

CONCLUSION

Applicant submits that all of the claims are in condition for allowance, which action is requested. If the Examiner finds that a telephone conference would expedite the prosecution of this application, please telephone the undersigned at the number provided.

The Commissioner is hereby authorized to charge any underpayment of fees associated with this communication, including any necessary fees for extensions of time, or credit any overpayment to Deposit Account No. 50-0815, order number ASIL-002CIP.

Respectfully submitted,
BOZICEVIC, FIELD & FRANCIS LLP

Date: Sept 28, 2004

By: _____
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